ADCY5 couples glucose to insulin secretion in human islets

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Abbreviations AC, adenylate cyclase; ADP, adenosine diphosphate; ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate; Epac, Exchange protein activated by cAMP; eQTL, expression quantitative trait locus; fMCI, functional multicellular calcium imaging; GIP, glucose-dependent insulino tropic polypeptide; GLP-1, glucagon-like peptide-1; GSIS, ISIS, glucose- or incretin-stimulated insulin secretion; K_{ATP}, ATP-sensitive K⁺ channel; shRNA, short hairpin RNA; TIRF, Total...
ABSTRACT

Single nucleotide polymorphisms (SNPs) within the ADCY5 gene, encoding adenylate cyclase 5, are associated with elevated fasting glucose and increased type 2 diabetes (T2D) risk. Despite this, the mechanisms underlying the effects of these polymorphic variants at the level of pancreatic beta cells remain unclear. Here, we show firstly that ADCY5 mRNA expression in islets is lowered by the possession of risk alleles at rs11708067. Next, we demonstrate that ADCY5 is indispensable for coupling glucose, but not GLP-1, to insulin secretion in human islets. Assessed by in situ imaging of recombinant probes, ADCY5 silencing impaired glucose-induced cAMP increases and blocked glucose metabolism towards ATP at concentrations of the sugar >8 mM. However, calcium transient generation and functional connectivity between individual human beta cells were sharply inhibited at all glucose concentrations tested, implying additional, metabolism-independent roles for ADCY5. In contrast, calcium rises were unaffected in ADCY5-depleted islets exposed to GLP-1. Alterations in beta cell ADCY5 expression and impaired glucose signalling thus provide a likely route through which ADCY5 gene polymorphisms influence fasting glucose levels and T2D risk, while exerting more minor effects on incretin action.
INTRODUCTION

Type 2 diabetes mellitus (T2D) is one of the foremost health challenges currently facing developed societies. This metabolic disease, which affects ~8.3% of the adult population worldwide (http://www.idf.org/diabetesatlas) (1), usually reflects a failure of the beta cell mass to adapt output to increased peripheral insulin resistance. The resulting hyperglycemia and dyslipidaemia lead to debilitating complications, ranging from kidney failure and blindness to cardiovascular disease and cancer (2). Although the maintenance of an adequate functional beta cell mass is critical to avoid the development of diabetes (3), the molecular basis of beta cell failure is still poorly understood (4).

The mechanisms underlying glucose-stimulated insulin secretion (GSIS) from single beta cells involve uptake of the sugar via specific glucose transporters (5), enhanced ATP synthesis (6) and closure of ATP-sensitive $K^+$ channels ($K_{ATP}$) (7). The consequent plasma membrane depolarization leads to $Ca^{2+}$ influx (8) and exocytosis from secretory granules (9), both of which are further potentiated by “$K_{ATP}$-independent” amplifying signals (10). In addition, incretin hormones such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP), released in response to food transit through the gut, potentiate insulin secretion in a glucose-dependent manner (11). Cognate receptor activation engages adenylate cyclases (ADCYs), enzymes which catalyze the generation of cAMP, a key intra- and inter-cellular signaling effector in the beta cell. Through its downstream interactions with protein kinase A (PKA) and Exchange protein activated by cAMP (Epac), cAMP drives changes including $Ca^{2+}$ influx, intracellular $Ca^{2+}$-mobilisation (12) and the enhanced fusion competence of secretory granules (13). By contrast, glucose evokes more modest increases in intracellular cAMP (14), possibly via the stimulation of
Ca²⁺-activated ADCYs such as ADCY1 and ADCY10 (15).

Both genetic (16) and environmental (4) risk factors conspire to determine the rate and extent of loss of insulin secretory capacity in T2D. Thus, the majority of genetic risk loci (~70) currently identified by genome-wide association (GWAS) or familial studies alter functional beta cell mass while exerting little, or occasionally a beneficial, effect on insulin sensitivity (17). Of note, recent genetic studies have provided evidence that several pathways converging on beta cell cAMP signaling may influence T2D risk. For example, carriers of the major A-allele at rs11708067, or the C-allele at the neighbouring SNP rs2877716, lying on chromosome 3 in intron 3 of the ADCY5 gene, have an increased odds-ratio of developing T2D (18). ADCY5 is a Ca²⁺-inhibited Type III adenylate cyclase (19;20) and risk allele carriers present with elevated fasting glucose (21), but not impaired insulinogenic index or AUC<sub>insulin/glucose</sub> 2 h post-oral glucose load (22-25). These data strongly imply that ADCY5 activity may be required for normal insulin release in response to glucose but not incretin, the latter largely accounting for the effects of oral glucose (26). Whether and how SNPs exert control over beta cell function by influencing ADCY5 expression remains, nonetheless, unclear.

Therefore, the aims of the present study were to: 1) establish a link between genotype and ADCY5 mRNA levels; 2) silence ADCY5 expression in human islets using specific shRNAs; and 3) use in situ imaging approaches and hormone release assays to establish the role of the cyclase in regulating beta cell responsiveness to glucose and incretin.
RESEARCH DESIGN AND METHODS

Human islet isolation

Human islets were isolated from deceased heart-beating donors at transplantation facilities in Oxford, Geneva, Pisa, Edmonton and Milan with the relevant national and local ethical permissions, including consent from next of kin where required, and cultured as described (27). All studies involving human tissue were approved by the National Research Ethics Committee (NRES) London (Fulham), REC # 07/H0711/114.

Mouse islet isolation

Female C57BL/6 mice, 8-12 weeks of age, were euthanized by cervical dislocation and pancreatic islets isolated by collagenase digestion, as described (28). Animal procedures were approved by the Home Office according to the Animals (Scientific Procedures) Act 1986 of the United Kingdom (PPL 70/7349).

ADCY5 Genotyping

DNA samples were genotyped for the SNP rs11708067 by RFLP analysis as the G allele generated a HhaI site. A 250 bp region flanking the locus was amplified by PCR using Phire polymerase (Thermo) with the following primers: TCCGGAAGGCAAACACAGCA and AGCCAGGCTGCACCCAAGTG. The products were digested with HhaI and resolved by agarose gel electrophoresis.

Lentiviral delivery of shRNA

Lentiviral particles carrying shRNA expression constructs against human ADCY5 were acquired from Sigma-Aldrich (Supplementary Table 1). Multiplicity of infection (MOI) was calculated using Turbo-GFP particles on the same backbone
Specificity of ADCY5 gene silencing was confirmed in both dissociated and intact islets, assuming 1000-2000 cells/islet for the latter. In all cases, lentiviral particles containing scrambled shRNA were used as controls and islets infected for 48-72 h.

**Generation of Adenoviral Epac2-camps**

cDNA encoding the cAMP sensor Citrine/Cerulean-Epac2-camps (29) was cloned into pShuttleCMV via HindIII and Xho1 sites before recombination with pAdEasy1 and virus production as described (6;30).

**Real-time PCR**

Relative mRNA abundance was quantified by quantitative reverse transcription PCR (qRT-PCR) using SYBR green (31). Primers (Supplementary Table 2) were designed using PerlPrimer (32), specificity validated using a dissociation curve, and amplification efficiency determined using a dilution series. Expression of each gene was normalised to cyclophilin A (Ppia) and N-fold change in mRNA expression versus control calculated using the $2^{-\Delta\Delta CT}$.

ADCY5 mRNA levels were measured for eQTL analysis by qRT-PCR of RNA from isolated human islets as described earlier (33). The expression level of ADCY5 relative to TATA box binding protein (TBP) was determined by qRT-PCR using Taqman primers and reagents (Supplementary Table 3), and the comparative Ct method ($2^{-\Delta\Delta CT}$) used for subsequent calculations.

**Immunohistochemistry**

Islets were fixed overnight at 4°C in paraformaldehyde before application of primary antibodies against ADCY5/6 (Abcam; cat no. ab66037) (34) and either
guinea pig anti-insulin 1:200 or mouse anti-glucagon 1:1000 (both DAKO). Revelation was performed with goat anti-rabbit Alexa-488 and either goat anti-guinea pig Alexa-568 or goat anti-mouse Alexa-488 antibodies (both 1:500, Invitrogen). Images were acquired as described (27).

**Measurements of insulin secretion from isolated islets**

Insulin secretion was measured from groups of 5-6 islets per well, incubated for 30 min in 0.5 ml of Krebs-HEPES-bicarbonate (KHB) solution (130 mM NaCl, 3.6 mM KCl, 1.5 mM CaCl₂, 0.5 mM MgSO₄, 0.5 mM NaH₂PO₄, 2 mM NaHCO₃, 10 mM HEPES, and 0.1 % (wt/vol) BSA, pH 7.4) at 37°C containing the indicated glucose and GLP-1 (7-36 human amide fragment) (Cambridge Bioscience) concentrations (35). Total islet proinsulin and insulin were measured after acid ethanol extraction and sonication using specific radioimmunoassays (RIA) (EMD Millipore).

**Live:dead and TUNEL assays**

Islets were incubated with 3 µM calcein-AM (Life Technologies) and 2.5 µM propidium iodide (PI; Sigma-Aldrich) before detection of absorbance/emission at 491/525nm and 561/620nm, respectively. The islet area occupied by dead cells was expressed as a unitary ratio *versus* that occupied by live cells. Apoptosis was assessed using a TUNEL staining kit (Promega) according to the manufacturer’s instructions and islets counterstained against insulin before detection of absorbance/emission at 491/525nm and 561/620nm, respectively.

**Calcium, ATP/ADP and cAMP imaging**

Isolated islets were incubated (37°C, 95% O₂/5% CO₂) for 1 h in fluo2-AM (10 µM) diluted in a bicarbonate buffer solution (120 mM NaCl, 4.8 mM KCl, 1.25
mM NaH₂PO₄, 24 mM NaHCO₃, 2.5 mM CaCl₂, 1.2 mM MgCl₂ and 3 mM D-glucose; all Sigma-Aldrich). Functional multicellular Ca²⁺ imaging was performed using a Nipkow spinning-disk head (27). A near identical distribution of Fluo-2 intensity was detected in Con and ADCY5 shRNA-treated islet under resting (3 mM glucose) conditions, indicating that basal Ca²⁺ levels were likely unaffected by gene knockdown, assuming similar dye loading (Supplementary Fig. 1).

For imaging of cytosolic ATP/ADP with Perceval (6) or cAMP with Epac2-camps, islets were infected for 48 h with adenoviruses at MOI 10-100, giving infection of the first 2-3 islet cell layers. Cells were imaged using a HEPES-bicarbonate buffer (120 mM NaCl, 4.8 mM KCl, 24 mM NaHCO₃, 0.5 mM Na₂HPO₄, 5 mM HEPES, 2.5 mM CaCl₂ and 1.2 mM MgCl₂). For Perceval, absorbance/emission was 491/525 nm. For the Epac2-camps probe, excitation was delivered at 440 nm and emitted signals captured using cerulean (530 nm) and citrine (470 nm) filters. FRET was calculated as the ratio of cerulean:citrine fluorescence and, for each experiment, expressed as a percentage of that obtained following maximal stimulation with 50 µM forskolin (FSK). No differences in probe responses to FSK were detected in control and ADCY5 shRNA-treated tissue (F/Fₘ𝑖𝑛 = 1.07±0.01 versus 1.06±0.01 AU, Con versus ADCY5, respectively; P>0.05).

ZIMIR imaging

ZIMIR, a membrane-bound probe which fluoresces upon binding of zinc (Zn²⁺) co-released with insulin from granules, was used to dynamically monitor insulin secretion as previously described (27;36). Briefly, islets were incubated with 10 µM ZIMIR for 2 h and imaged in bicarbonate buffer supplemented with 1 µM EGTA. Following acquisition (absorbance/emission = 491/525nm), islets were
divided into 20 sub-regions before extraction of intensity over time traces and analysis of amplitude and AUC.

**Total internal reflection of fluorescence (TIRF) microscopy**

Insulin-stained PFA-fixed islets were subjected to TIRF imaging to capture a super-resolution snapshot of sub-membrane insulin granule distribution. The evanescent field was produced using a 561nm laser (CrystaLaser) and a 100x 1.6NA objective (Zeiss). Insulin granule density was expressed as a unitary ratio versus the total membrane area, the latter being clearly visible between adjacent cells.

**Correlation and frequency analysis**

Correlation and frequency analyses were performed as previously detailed (27;37). Briefly, intensity over time traces were extracted for each fluo-2-loaded cell using a region of interest (ROI) before manual triage for glucose-responsiveness based on rises above a 25% threshold. The Pearson product moment correlation was performed for all possible cell pair combinations and significance (P<0.05) calculated versus the expected t-distribution of independent R-values. Functional connectivity maps showing the location of significantly correlated cell pairs were then constructed based upon correlation strength and position within the imaged field (x-y). Phase maps were compiled by converting the normalised intensity of each cell to a value between 1-100% and assigning this to a color using a light-dark ramp. Ca\(^{2+}\)-spiking frequency was measured using the Fast Fourier Transform (FFT).

**Statistical Analysis**

Data distribution was determined using D’Agostino omnibus or Shapiro-Wilk tests. Non-multifactorial pairwise comparisons were made using Mann-Whitney U-test or Student’s unpaired and paired t-tests. Two-way analysis of variance (ANOVA)
was used to assess interactions between multiple treatments (P<0.01) and pairwise comparisons performed using Bonferonni post-tests. Expression quantitative trait locus (eQTL) data were stratified according to genotype, BMI, age and gender. Factors included in the model were selected using the Akaike Information Criterion (AIC), resulting in the exclusion of age and BMI as these were uninformative with regards to ADCY5 expression. To account for any prediction error, linear regression analyses were performed separately for both excluded variables, yielding non-significant relationships (P>0.05). Due to a significant interaction between gender and genotype in donors 22-70 years of age (P = 0.0476; two-way ANOVA), effects of genotype on mRNA abundance were assessed within gender group using one-way ANOVA. In all cases, analysis was performed using R (R Project), Graphpad Prism (Graphpad Software), IgorPro (Wavemetrics) and MATLAB (Mathworks), and results considered significant at P<0.05.
RESULTS

ADCY5 is expressed in human islets at the mRNA and protein level

We sought firstly to characterise ADCY5 expression in tissue isolated from human and murine donors. Confirming previously published microarray data (33), SYBR Green qRT-PCR analyses revealed that ADCY5 and ADCY6 transcript levels were similar in human but not mouse islets (Fig. 1A). Immunohistochemistry (IHC) using an antibody against ADCY5, and with some reported cross-reactivity against ADCY6, demonstrated the localisation of both enzymes in the cytoplasm of insulin and glucagon immunopositive cells throughout individual human islets (Fig. 1B).

ADCY5 mRNA levels are influenced by genotype

While GWAS provides statistically powerful information concerning associations between SNPs and T2D risk, it is unable to report on how the expression of genes at variant loci are altered in tissues implicated in glucose homeostasis. Since the elevated glucose levels associated with T2D may influence ADCY5 expression, we attempted to correlate genotype at SNP rs11708067 with ADCY5 mRNA abundance using tissue from a catalogue of healthy donors. Allele frequency was close to that expected from previous studies (~75% A) (18), and Taqman qRT-PCR analysis of RNA from isolated islets revealed ~2-fold lower mean ADCY5 expression in male AA versus AG carriers under 70 years of age (Fig. 1C) (Supplementary Table 4). When considered separately, neither age nor BMI significantly influenced ADCY5 expression for either genotype (Fig. 1D and E).
ADCY5 is required for glucose-stimulated insulin secretion

Given the above association between ADCY5 expression and fasting glucose levels and genotype in man, we next explored a role for this gene in human beta cell stimulus-secretion coupling. Short hairpin RNAs (shRNA) directed against various sequences of the ADCY5 gene were delivered into dispersed cells or islets using replication-incompetent lentiviruses (n = 26 separate normoglycemic donors, see Supplementary Table 5). Gene silencing efficiency was determined using qRT-PCR (Fig. 2A and B) and effects confirmed at the protein level using IHC and an antiserum raised against ADCY5/6 (Fig. 2C). Although mRNA levels were less affected by shRNA in intact than dissociated islets, this was most likely due to limited penetration of virus into the islet core (38); cells within the imaged layers were nevertheless typified by the near-complete absence of ADCY5 immunoreactivity. ADCY6 mRNA abundance was not altered by ADCY5 silencing, suggesting that the action of the shRNA on immunoreactivity was largely due to loss of the latter mRNA message (Fig. 2D). Excluding any potential effects of impaired cAMP signaling on beta cell survival, cell viability and apoptosis indices were normal (Fig. 2E and F).

The impact of ADCY5 silencing on glucose- or incretin-stimulated insulin secretory dynamics was subsequently assessed using Nipkow spinning disk microscopy (27) to image zinc co-released from insulin-containing granules within individual ZIMIR-stained islets (27;36). The amplitude and area under the curve (AUC) of glucose (11mM)-stimulated insulin release were markedly impaired in islets silenced for ADCY5 (Fig. 2G). By contrast, insulin release dynamics in response to GLP-1 were subtly improved in tissue depleted for ADCY5, suggesting the presence of an intact incretin axis (Fig. 2H). The observations with ZIMIR were confirmed using conventional static incubation techniques followed by
radioimmunoassay of supernatant, and we further detected no significant effect of gene silencing on KCl-stimulated insulin release (Fig 2I-K). Donor variability was largely accounted for by a paired experimental design, and this was further supported by linear regression analyses which revealed no relationship between age, BMI and the magnitude suppression of glucose-stimulated insulin release in ADCY5-silenced islets (Supplementary Fig. 2). Insulin content was similar in control and ADCY5 shRNA-treated islets, although there was a tendency towards an increased proinsulin:insulin ratio in the latter (Table 1), as expected from studies in patients harboring SNPs in or near the ADCY5 locus (25). Lastly, and in line with the secretory measures, TIRF imaging of insulin-stained islets confirmed that ADCY5 knockdown did not alter the distribution of granules located in vicinity of the plasma membrane (Supplementary Fig. 3 A and B).

**ADCY5 couples glucose to cAMP generation, Ca\(^{2+}\) rises and beta cell connectivity**

Using Epac2-camps (29) to measure cAMP rises in beta cells (Supplementary Fig. 4), almost a three-fold reduction in glucose- but not FSK-stimulated cAMP generation could be detected in ADCY5-silenced islets (Fig. 3A and B). Since cAMP elevations are linked to enhanced electrotonic coupling (39), the effects of ADCY5 depletion on the cell-cell communication processes underlying the intraislet regulation of insulin secretion were next investigated. Cytosolic free Ca\(^{2+}\) levels were thus used as a proxy to measure the electrical dynamics which orchestrate Ca\(^{2+}\)-dependent hormone secretion from the hundreds of cells residing within the first few microorgan layers; under the conditions used, Ca\(^{2+}\) changes chiefly reflect those within beta cells (27). In line with its dramatic effects on secretion, ADCY5 silencing suppressed both the amplitude and AUC of glucose-evoked Ca\(^{2+}\) rises throughout the imaged
population (Fig. 3C-E; Supplementary movies 1 and 2) \( (n = 3\) donors; BMI range = 25.4-27.4; age range = 52-76). Furthermore, connectivity between individual beta cells, analysed by large-scale mapping of long term (~30 min) evolutions in correlated cell-cell interactivity (40), and recently shown to be a key element in the insulin secretory response (27;41), was also reduced in ADCY5-silenced islets (Fig.3F and G). Neither the number of responsive cells (Fig. 3H) nor the frequency of \([\text{Ca}^{2+}]_i\) oscillations (Fig. 3I) were affected by ADCY5 knockdown, suggesting that the absence of the cyclase was unlikely to impair insulin release by targeting the rhythmicity of a beta cell subpopulation. Implying that the effects of silencing were unlikely to reflect a long-term consequence of ADCY5 depletion, a similar degree of suppression of glucose-induced \(\text{Ca}^{2+}\) rises was obtained using 20 \(\mu\text{M}\) NKY80, a relatively selective chemical inhibitor of the enzyme (IC\(_{50}\) = 8.3 \(\mu\text{M}\), 132 \(\mu\text{M}\) and 1.7 mM for ADCY5, 3 and 2, respectively) (42) (Supplementary Figure 5A). Demonstrating the specificity of both shRNA and NKY80, the inhibitory actions of the drug were lost following ADCY5 silencing (Supplementary Figure 5B).

**GLP-1-stimulated beta cell activity does not involve ADCY5**

Consistent with the lack of effect of gene silencing on GLP-1-induced insulin release, loss of ADCY5 failed to impact \(\text{Ca}^{2+}\) changes, and even appeared to augment \(\text{Ca}^{2+}\) increases in response to the incretin without modulating the proportion of responsive cells (Fig. 4A and B; Supplementary movies 3 and 4). Furthermore, GLP-1-mediated increases in beta cell-beta cell connectivity, critical for generating the acute (5-10 min) bursts in coordinated activity which underlie incretin potentiation of glucose-stimulated insulin secretion (27), was unchanged following treatment with anti-ADCY5 shRNA (Fig. 4C-E). In line with this, GLP-1R mRNA expression levels were unaffected by ADCY5 silencing (Fig. 4F).
Glucose still increases cytosolic ATP/ADP ratio following ADCY5 silencing

We wondered whether the substantial reductions in glucose-induced Ca\textsuperscript{2+} rises and insulin release, detected in ADCY5-silenced islets, were accompanied by fulminant changes to beta cell glucose metabolism. To record ATP dynamics specifically in beta cells in real-time, the expression of the recombinant probe Perceval was directed in human islets using an adenoviral vector (6;43). Confirming tropism of the virus for beta cells, as previously described in rodent islets (44), GFP fluorescence was restricted to insulin-immunopositive cells (Fig. 5A). In response to increasing glucose concentrations (3-17 mM), beta cells responded with large, coordinated and non-oscillatory elevations in ATP/ADP ratio (Fig. 5B). Although significantly reduced, a glucose-induced increase in ATP/ADP ratio could still be detected following ADCY5 silencing (Fig. 5C). Further excluding a role for cAMP-independent effects on K\textsubscript{ATP} or VDCC activity, ADCY5 silencing did not alter Ca\textsuperscript{2+} rises induced by KCl applied in the absence and presence of the ATP-sensitive K\textsuperscript{+}-channel opener, diazoxide (Supplementary Figure 6A and B). Consistent with our previous findings in MIN6 beta cells (12), GLP-1 was able to provoke significant rises in ATP/ADP ratio under both low (3 mM) and high (16.7 mM) glucose conditions (Fig. 5D-F), as well as in the presence of ADCY5-silencing (Fig. 5G and H). Hence, an action of glucose to accelerate oxidative metabolism towards ATP synthesis is not a prerequisite for GLP-1 signaling.

To determine the relative contribution of metabolism to ADCY5-regulated beta cell function, ATP/ADP dynamics were imaged following exposure to increasing glucose concentrations. Whereas both control and ADCY5-silenced islets responded normally to 5 and 8mM glucose (Figure 6A), the latter failed to respond to further elevation of the sugar, and this deficit could be rescued using forskolin to elevate
cAMP (Figure 6B). Cytosolic Ca$^{2+}$ responses to 8mM glucose, however, remained suppressed following $ADCY5$ knockdown (Figure 6C).
DISCUSSION

The aim of the current study was to explore the role of ADCY5 in the regulation of insulin secretion from human islets. We show that ADCY5 is required to link glucose-derived signals to the intracellular Ca\(^{2+}\) rises which principally drive insulin granule exocytosis. By contrast, incretins such as GLP-1 remain competent to evoke insulin release in the face of ADCY5 silencing (Scheme Fig. 7). Providing compelling evidence that defective ADCY5 action may contribute to impaired fasting glucose were the observations that mRNA expression was reduced in islets from subjects harbouring risk alleles at rs11708067. Interestingly, ADCY5 mRNA levels were also reduced in beta cells from (un-genotyped) patients with T2D versus healthy donors (33), providing a further link with disease status.

Assessed by next generation sequencing (RNA-Seq), ADCY5 mRNA occupies the top 13th centile of all mRNAs in human islets (45) and is the most strongly expressed member of the ADCY family in this tissue, with mRNA levels ~2-fold higher than those of ADCY1, the next most abundant isoform, and 50% higher than ADCY6 mRNA. Assuming similar levels of PPIA mRNA, our qRT-PCR results in isolated human islets are broadly consistent with this observation (Fig. 1A). Of note, however, analysis of laser-capture dissected beta cells (33) (Gene Expression Omnibus public repository, Accession GSE20966) and a beta cell enriched-fraction from human islets (46) revealed lower levels of ADCY5 mRNA (~50th and ~65th centiles, respectively), being in each case ~50% of those of ADCY6. Interestingly, in the present study, Adcy5 mRNA was barely detectable in mouse islets, being expressed at a level ~40-fold lower than Adcy6 (Fig. 1). A similar, though less marked (~8 fold), preponderance of ADCY6 over ADCY5 mRNA also exists for rat islets (47), demonstrating marked species-variability in the relative abundance of ADCY
isoforms within this tissue. Since these variations do not appear to reflect the greater proportion of alpha cells in human (48) versus rodent (49) islets, ADCY5 may serve a non-conserved function between mammalian species.

Levels of mRNA encoding ADCY8, previously reported to be regulated by glucose in rat and human islets (50), were recently shown to be much lower in human islets than either ADCY5 or -6 (45). Confirming these findings, ADCY8 mRNA was undetectable in 4 out of the 5 independent repeats we conducted in human islets, and in the single experiment in which an amplicon was present, C_T values were incompatible with accurate quantification (37.05 ± 0.49 cycles).

Of other genes lying 250 kb either side of rs2877716 and rs11708067, which might also conceivably mediate the observed effects on diabetes risk, MYLK transcripts are barely detectable in human islets (45;46). On the other hand, SEC22A, PDIA and PTPLB are each detectably expressed, and so may contribute. Nonetheless, our observations on the effects of ADCY5 silencing are entirely consistent with the observed phenotype of subjects possessing risk alleles (22).

The strong dependence of glucose-stimulated insulin secretion upon ADCY5 expression in human islets is surprising given the abundant presence of ADCY6, and the redundancies inherent to the cAMP signaling cassette (51). Although the above differences at the mRNA level are not necessarily reflective of protein quantity or enzymatic activity, the observed sensitivity to ADCY5 depletion may reflect either micro-compartmentalization of ADCY5-containing complexes in proximity to Ca^{2+} influx and release sites (52), or a requirement for ADCY5 in cAMP-independent signaling processes. Thus, while our data indicate that ADCY5 couples glucose to insulin secretion by translating a glucose signal into cAMP generation (53), GLP-1
receptors may engage alternative ADCY family members to modify cAMP dynamics. Further studies will be required to explore the latter possibility (Scheme Fig. 7). Of note, global deletion of ADCY5 in mice leads to longevity and the resistance of cardiomyocytes to oxidative stress via the up-regulation of Ras-MAPK signaling (20). A similar mechanism might therefore contribute to the small but significant enhancement of GLP-1 signaling observed here in human beta cells after ADCY5 silencing.

Although ADCY5 is Ca\(_{2+}\)-inhibited *in vitro* (19), the range of Ca\(_{2+}\) concentrations over which inhibition occurs (>10 µM) comfortably exceeds normal intracellular concentrations of these ions in beta cells (8). Whether ADCY5 is therefore a direct target for activation by intracellular signals generated by glucose (ATP, etc), or is rather a passive but essential element of a glucose-activated signaling pathway leading to Ca\(_{2+}\) influx and insulin release, remains to be established.

Recent studies have shown that the intra-islet regulation of cell-cell communication is critical for the proper generation of hormone release following secretagogue challenge (27). Providing further evidence that the islet context is an important player in insulin secretion was the observation in the present study that beta cell responses to glucose were less coordinated in islets depleted of ADCY5. While the mechanisms underlying this phenomenon remain obscure, cAMP has been shown to alter beta cell gap junction conductance (39), and the resultant enhanced intercellular coupling may contribute to the insulin-raising actions of cAMP-elevating agents such as glucose and incretins. Whereas we recently showed that knockdown of connexin 36 markedly reduced coordinated cell responses to incretin, more modest effects on glucose action were observed (27). The impact of cAMP on glucose-induced cell connectivity may therefore also stem from perturbed paracrine signaling
circuits between beta-, alpha- and other cell types, in addition to enhanced
electrotonic coupling. Likewise, an intriguing possibility is that ADCY5 suppression
may also affect glucagon and, potentially, GLP-1 (54) secretion from neighboring
alpha cells to affect beta cell “glucose competence”. In any case, ADCY5-depleted
islets still displayed impaired Ca\textsuperscript{2+} responses even when metabolic dysfunction was
accounted for (Fig. 6B), supporting the view that other downstream processes,
including cell-cell communication, are targeted by cAMP signaling.

Remarkably, GLP-1 evoked Ca\textsuperscript{2+} rises and hormone release even when
glucose-stimulated insulin secretion was abolished by ADCY5 silencing. This implies
that GLP-1 signaling - generally believed to hinge on increased intracellular cAMP
levels (55) - is sufficient under these conditions to elicit closure of K\textsubscript{ATP} and induce
Ca\textsuperscript{2+} influx and exocytosis. Indeed, studies by Seino et al (56) have demonstrated that
GLP-1 is still able to evoke insulin secretion in the complete absence of K\textsubscript{ATP}
channels. Thus, the glucose-dependency of GLP-1-induced insulin secretion is
unlikely solely to reflect the effects of the sugar on oxidative metabolism and hence
the closure of K\textsubscript{ATP} channels, especially in light of data showing the absence of GLP-
1-induced Ca\textsuperscript{2+} rises in human islets incubated at non-permissive glucose
concentrations (27). Rather than a simple summation of glucose and GLP-1-derived
Ca\textsuperscript{2+} signals, the ATP/ADP responses to the latter, readily detectable at low glucose
concentrations, may instead be converted into a depolarising stimulus through a
complex interplay between K\textsubscript{ATP}-dependent and independent pathways (10).

In summary, we describe here a novel role for the GWAS-identified gene
ADCY5 in the normal regulation of insulin secretion from human islets of Langerhans.
Thus, we identify a pathway that converts ADCY5 gene polymorphisms into defective
beta cell function. Since beta cell decompensation is a hallmark of T2D pathogenesis
irrespective of genotype, ADCY5 may provide a useful target for the restoration of insulin release in man.
|                          | Con      | shRNA    |
|--------------------------|----------|----------|
| Proinsulin (PI) content (ng/islet ± SEM) | 0.82 ± 0.11 | 0.93 ± 0.07 |
| Insulin (I) content (ng/islet ± SEM)    | 32.8 ± 3.5 | 29.8 ± 3.1  |
| PI:I (%)                         | 2.6 ± 0.5 | 4.0 ± 1.2  |

**Table 1:** Insulin and proinsulin content in control and ADCY5 shRNA-treated islets (n = 4 donors).
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AUTHOR CONTRIBUTIONS

D.J.H. and G.A.R. jointly supervised the research. G.A.R., D.J.H. and R.K.M. conceived and designed the study. D.J.H., R.K.M., L.M., T.J.P. S.G.B. and F.S. performed the experiments. D.J.H., R.K.M., L.M., T.J.P. and G.A.R. performed analysis. D.L., W-H.L., K.L.E. and D.M.F.C. provided reagents. M.B., P.M., V.L., D.B., L.P., P.J., S.J.H. and A.M.J.S. isolated and provided human islets. G.A.R. and D.J.H. wrote the paper with input from all the authors.
G.A.R. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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FIGURE LEGENDS

Figure 1: ADCY5 is expressed in isolated human islets and affected by T2D risk alleles. (A) ADCY5 and ADCY6 are expressed at similar levels in human islets (n = 4 donors). Conversely, Adcy6 mRNA expression is ~40-fold more abundant than that of Adcy5, which is barely detectable in mouse islets (n = 3 female and 3 male animals; **P<0.01 versus ADCY5, Student’s t-test). (B) Immunostaining using an anti-ADCY5 immunoglobulin, with some reported cross-reactivity to ADCY6, reveals the cytoplasmic distribution of both proteins throughout the human alpha- and beta-cell populations (DAPI, blue; scale bar, 60 µm). (C) Scatter plot showing reduced ADCY5 mRNA abundance in islets from males under 70 years of age who are carriers of the AA risk allele at rs11708067 (*P<0.043 versus AG; one-way ANOVA; n = 7 donors for each allele). Values represent mean ± SEM. (D) Age of donors is not significantly correlated with ADCY5 mRNA expression (R^2 = 0.21 and R^2 = 0.01 for AG and AA, respectively; linear regression) (P values shown on graph). (E) As for (D) but BMI (R^2 = 0.23 and R^2 = 0.003, AG versus AA, respectively; linear regression) (P values shown on graph).

Figure 2: ADCY5 silencing inhibits glucose- but not GLP-1-stimulated insulin secretion. (A-B) Lentivirus harboring shRNA against ADCY5 reduces expression by >50% in both dispersed and intact islets (**P<0.01 versus control (Con); Student’s paired t-test; n = 3-4 donors). (C) ADCY5/6 protein expression is markedly reduced in the first few cell layers of intact islets, as determined using confocal imaging (n = 6 islets from two donors). (D) ADCY6 mRNA expression is unaffected by ADCY5 silencing (NS, non-significant versus Con; Student’s paired t-test; n = 4 donors). (E) Dead:live cell ratio is identical in control and shRNA-treated islets (positive control, Triton X-100; NS, non-significant versus Con; Mann-Whitney U-test; n = 10-11 islets from three donors). (F) As for (E) but TUNEL assay for apoptosis (n = 9 islets from three donors). The proportion of apoptotic beta cells was expressed as a fraction area versus non-apoptotic insulin positive cell mass (Vv). (G) ADCY5 knockdown suppresses glucose-induced insulin secretory dynamics, as shown by bar graphs of area-under-the-curve (AUC) and amplitude of ZIMIR responses (mean traces, left panel; n = 8-9 islets from four donors). (H) GLP-1-stimulated insulin secretory dynamics are subtly improved following ADCY5 depletion (mean traces, left panel;
AUC and amplitude, right panel; \( n = 7 \) islets from three donors) (NS, non-significant). (I) Glucose-stimulated insulin release into static culture is impaired in shRNA-treated islets, as determined using radioimmunoassay (G3 and G16.7, 3 mM and 16.7 mM glucose, respectively) \( (n = 4-8 \) donors). KCl 30 mM was added as a control. (J) As for (I) but stimulation index \textit{versus} 3 mM glucose to better account for the variation between islet preparations \( (*P<0.02 \text{ versus } \text{Con at } 16.7 \text{ mM glucose}; \text{Mann-Whitney U-test}). \) (K) As for (J) but \textit{versus} 16.7 mM glucose (G16.7) \((*P<0.01 \text{ versus } 16.7 \text{ mM glucose for each group}; \text{Mann-Whitney U test}). \) Values represent mean ± SEM.

\textbf{Figure 3: ADCY5 depletion suppresses glucose-induced increases in cytosolic free \( \text{Ca}^{2+} \).} (A) ADCY5 silencing decreases cytosolic cAMP levels as determined using the recombinant probe Epac2-camps (FSK, forskolin) (representative traces shown; gray/black, smoothed; red, raw). (B) As for (A) but summary data showing a reduction in measured FRET signal \textit{versus} maximal stimulation with FSK (%), as well as decreased AUC \((**P<0.01 \text{ versus } \text{Con}; \text{Student’s t-test}; n = 12 \text{ recordings from three donors}). \) (C) ADCY5 knockdown suppresses 11mM glucose (G11)-evoked cytosolic \( \text{Ca}^{2+} \) rises (left panel; mean traces) (right panel; zoom-in of \( \text{Ca}^{2+} \) oscillations). (D) AUC and amplitude of \( \text{Ca}^{2+} \) rises are reduced in shRNA-treated islets (right panel; \( **P<0.01 \text{ versus } \text{Con}; \text{Mann-Whitney U-test}; n = 10 \text{ islets from three donors}). \) (E) Pseudocolored control- and shRNA-treated human islets during exposure to 11 mM glucose (recording time = 40 min; image cropped to display a single islet). (F) ADCY5 is required for long term evolutions in coordinated cell activity following exposure to elevated glucose \((**P<0.01 \text{ versus } \text{Con}; \text{Mann-Whitney U-test}; n = 9-10 \text{ islets from three donors})\) (correlation calculated over 20-30 min). (G) Representative functional connectivity map depicting location, number and strength (color-coded; 0 [blue] = lowest, 1 [red] = highest) of significantly correlated cell pairs (Pearson \( R \) coefficient, \( P<0.05 \)). Note that ADCY5 silencing decreases both the number and strength of correlations. (H) Gene-silencing does not significantly alter the percentage (%) of glucose (11 mM)-responsive cells (NS, non-significant \textit{versus} \text{Con}; \text{Mann-Whitney U-test}). (I) The cumulative distribution of \( \text{Ca}^{2+} \)-spiking frequencies remains identical in control- and shRNA-treated islets. Values represent mean ± SEM.
**Figure 4:** ADCY5 does not mediate GLP-1-stimulated cytosolic Ca\(^{2+}\) increases. (A) ADCY5 knockdown subtly improves GLP-1-responses (left panel, mean traces), as evidenced by increased AUC and amplitude of cytosolic Ca\(^{2+}\) rises in shRNA-treated islets (right panel; **P<0.01 versus Con; Mann-Whitney U-test; n = 10 islets from three donors). (B) The proportion of GLP-1-responsive cells is similar in control- and shRNA-treated islets (NS, non-significant versus Con; Mann-Whitney U-test). (C) ADCY5 silencing does not affect coordinated beta cell responses to 11mM glucose plus GLP-1 (representative Ca\(^{2+}\) traces; top panel; gray, smoothed; red, raw) (heatmap depicting min-max for each cell; bottom panel) (n = 10 islets from three donors; correlation measured using 5 min windows). (D) Histogram showing mean % significantly correlated cell pairs in control- and shRNA-treated islets before, during and after GLP-1 application (NS, non-significant; two-way ANOVA). (E) Representative weighted graphs demonstrating large increases in beta cell connectivity following exposure to GLP-1 in both normal and ADCY5-depleted islets (scale bar, 50 µm). (F) Gene-silencing does not alter GLP-1R mRNA expression (NS, non-significant versus Con; Student’s paired t-test; n = 3 donors). Values represent mean ± SEM.

**Figure 5:** ADCY5 alters beta cell energetics. (A) Expression of the ATP/ADP probe Perceval is predominantly restricted to beta cells, as shown using immunohistochemistry with antibodies against insulin and glucagon (scale bar, 25 µm top panels and 20 µm bottom panels). (B) Glucose (17 mM) (G17) is still able to induce increases in ATP/ADP ratio following ADCY5 silencing (representative traces shown; gray/black, smoothed; red, raw) (C) Bar graph showing a significant effect of shRNA treatment on the amplitude of ATP/ADP rises in response to 17mM glucose (*P<0.05 versus Con; Mann-Whitney U-test; n = 9-10 islets from three donors). (D) GLP-1 increases ATP/ADP in the presence of permissive (17 mM) glucose concentrations (representative traces shown; gray/black, smoothed; red, raw). (E) As for (e) but in the presence of non-permissive (3mM) glucose concentrations. (F) Summary statistics demonstrate similar effects of GLP-1 on ATP/ADP in islets exposed to 3 or 17 mM glucose (NS, non-significant versus Con; Mann-Whitney U-test; n = 10-13 recordings from five donors). (G) ATP/ADP responses to GLP-1 are similar in control and ADCY5 shRNA-treated islets (representative traces shown; gray/black, smoothed; red, raw). (H) Summary statistics demonstrate no significant
effect of gene-silencing on GLP-1-induced ATP/ADP rises (NS, non-significant versus Con; Mann-Whitney U-test; \(n = 6\) recordings from two donors). Values represent mean ± SEM.

**Figure 6: ADCY5 targets non-metabolic processes to alter \(Ca^{2+}\) responses.** (A) Impaired ATP/ADP responses are only present in ADCY5-silenced islets at glucose concentrations of 11 mM and above (**P<0.01 versus Con; two-way ANOVA; \(n = 9\) recordings from three donors). (B) Elevation of cAMP using forskolin (FSK) rescues ATP/ADP rises in ADCY5-silenced islets following transition from 3 mM to 17 mM glucose (G3-G17) (**P<0.01 versus shRNA; one-way ANOVA; \(n = 9\) recordings from three donors; \(n = 4-5\) recordings). (C) ADCY5 knockdown suppresses beta cell \(Ca^{2+}\) responses following transition from 3 mM to 8 mM glucose (G3-G8) (left panel, representative traces; gray/black, smoothed; red, raw), as evidenced by reduced amplitude (right panel) (**P<0.01 versus shRNA; Mann-Whitney U-test; \(n = 8-9\) recordings from three donors).

**Figure 7: Schematic of ADCY5 function in human beta cells.** Glucose-stimulated insulin secretion (GSIS) relies on \(K_{ATP}\)-dependent and -independent signals. The latter include cAMP generation and this likely requires ADCY5 activation by the sugar to increase ATP generation, \(Ca^{2+}\) influx and exocytosis (left panel). By contrast, incretins such as GLP-1, believed primarily to engage cAMP-signaling pathways, may potentiate insulin secretion via other ADCY isoforms (right panel).
**A**

Human

Mouse

Diabetes

mRNA/ppia

ADCY5

ADCY6

0.00

0.05

0.10

0.15

0.20

0.25

mRNA/ppia

**B**

Insulin

ADCY5/6

Merge

Glucagon

ADCY5/6

Merge

**C**

Normalised ADCY5 expression

AG

AA

Allele

**D**

Normalised ADCY5 expression

Age (years)

P=0.90

P=0.58

P=0.89

P=0.58

**E**

Normalised ADCY5 expression

BMI

P=0.89

P=0.58

P=0.58

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**Fig. 2**

Dispersed (A) and Islet (B) cells show different responses to insulin stimulation. **C** and **D** illustrate the fold-change in ADCY5 expression (Con: 1.0). In **E**, the dead/live ratio is shown to be unaffected by insulin treatment. **F** displays the beta cell apoptosis, with no significant difference between control and shRNA. **G** and **H** show ZIMIR F/Fmin response to G3 and G11, respectively, with GLP-1 treatment showing a significant increase in ZIMIR F/Fmin over time (AUC (AU)). **I-J** and **K** highlight the differences in insulin production and stimulation index between G3, G16.7, GLP-1, and KCl treatments, with insulin production being significantly higher in G16.7 and GLP-1 treatments compared to G3.

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**Fig. 3**

A. G3 G20 FSK

Con shRNA

35%

5 min

B. ΔcAMP (% max)

|          | Con shRNA | Con shRNA |
|----------|-----------|-----------|
|          | 0         | 0         |

|          | 20        | 20        |
|----------|-----------|-----------|
|          | 40        | 40        |
|          | 60        | 60        |
|          | 80        | 80        |
|          | 100       | 100       |

AUC (AU)

C. Ca\(^{2+}\)/F/Fmin (AU)

Con shRNA

D. Amplitude (% baseline)

|          | Con shRNA | Con shRNA |
|----------|-----------|-----------|
|          | 100       | 100       |
|          | 110       | 110       |
|          | 120       | 120       |
|          | 130       | 130       |

Ca\(^{2+}\)/F/Fmin (AU)

E. Percentage cells (%)

F. ΔcAMP (% max)

|          | Con shRNA | Con shRNA |
|----------|-----------|-----------|
|          | 35%       | 35%       |

G. Frequency (mHz)

|          | Con shRNA | Con shRNA |
|----------|-----------|-----------|
|          | 0         | 0         |
|          | 20        | 20        |
|          | 40        | 40        |
|          | 60        | 60        |
|          | 80        | 80        |
|          | 100       | 100       |

H. Percentage cells (%)

|          | Con shRNA | Con shRNA |
|----------|-----------|-----------|
|          | NS        | NS        |

I. Percentage cells (%)
|                | Insulin | Diabetes | Glucagon |
|----------------|---------|----------|----------|
| **Figure 5**   |         |          |          |

**B**

| Time | G3 | G17 |
|------|----|-----|
| 5 min|    |     |
| F/Fmin | 0.1 AU | 0.1 AU |

**C**

| Δ[ATP]cyt (AU) | Con | shRNA |
|----------------|-----|-------|
|                | 0.20 | *     |

**D**

| Time | G17 | G17 + GLP-1 |
|------|-----|-------------|
| 15 min|     |             |
| F/Fmin | 0.05 AU | 0.05 AU |

**E**

| Time | G3 | G3 + GLP-1 |
|------|----|------------|
| 15 min|    |            |
| F/Fmin | 0.05 AU | 0.05 AU |

**G**

| Time | G17 | G17 + GLP-1 |
|------|-----|-------------|
| 10 min|     |             |
| F/Fmin | 0.07 AU | 0.07 AU |

**F**

| Δ[ATP]cyt (AU) | G3 | G17 | GLP-1 |
|----------------|----|-----|-------|
|                | +  | -   | +     |

**H**

| Δ[ATP]cyt (AU) | Con | shRNA |
|----------------|-----|-------|
|                | NS  |      |

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**Fig. 6**

A) Plot showing the change in Δ[ATP]_{cyt} (AU) with varying glucose concentrations (5-20 mM). The gray line represents the control (Con) and the black line represents ADCY5. Significant differences are indicated by ** and ***.

B) Bar graph depicting the change in Δ[ATP]_{cyt} (AU) for different conditions: Con, shRNA, and shRNA + FSK. The data are presented for G3-G17 and G3-G8 regions.

C) Graph showing the F/F_{min} (AU) over 5 minutes. The black line represents the control (Con) and the red line represents the shRNA condition. The region G3-G8 is highlighted.

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SUPPLEMENTARY INFORMATION

Supplementary Figure 1: Distribution of Fluo-2 basal intensity values is similar in Con and ADCY5 shRNA-treated islets.

Supplementary Figure 2: Donor age and BMI do not alter the magnitude suppression of glucose-stimulated insulin release following ADCY5-silencing.

Supplementary Figure 3: ADCY5-silencing does not alter insulin granule distribution near the plasmamembrane.

Supplementary Figure 4: Epac2-camps expression and localization in human islets.

Supplementary Figure 5: Effects of ADCY5-silencing can be mimicked using selective inhibitors of ADCY5 activity.

Supplementary Figure 6: ADCY5 does not alter Ca\textsuperscript{2+} responses to depolarisation.

Supplementary Movie 1: Timelapse recording of Ca\textsuperscript{2+}-responses to 11 mM glucose in a control-treated human islet.

Supplementary Movie 2: Timelapse recording of Ca\textsuperscript{2+}-responses to 11 mM glucose in a shRNA-treated human islet.

Supplementary Movie 3: Timelapse recording of Ca\textsuperscript{2+}-responses to 20 nM GLP-1 in a control-treated human islet.

Supplementary Movie 4: Timelapse recording of Ca\textsuperscript{2+}-responses to 20 nM GLP-1 in a shRNA-treated human islet.

Supplementary Table 1: shRNA sequences against ADCY5.

Supplementary Table 2: qRT-PCR primer sequences used for SYBR Green assays.

Supplementary Table 3: qRT-PCR primers for eQTL.

Supplementary Table 4: Characteristics of donors used for eQTL analysis.

Supplementary Table 5: Characteristics of donors used for non-eQTL studies.
**Supplementary Figure 1:** Distribution of Fluo-2 basal intensity values is similar in Con and ADCY5 shRNA-treated islets. The cumulative frequency of Fluo-2 intensity values (AU, arbitrary units) at 3 mM glucose is not significantly altered by ADCY5 knockdown (P>0.05; two-way ANOVA).
Supplementary Figure 2: Donor age and BMI do not alter the magnitude suppression of glucose-stimulated insulin release following ADCY5-silencing. (A) Age of donors is not significantly correlated with the percentage suppression of insulin secretion in ADCY5-silenced islets ($R^2 = 0.0001$; linear regression) (P values shown on graph). (B) As for (A) but BMI ($R^2 = 0.05$; linear regression) (P values shown on graph).
Supplementary Figure 3: ADCY5 silencing does not alter insulin granule distribution near the plasmamembrane. (A) TIRF imaging reveals similar insulin granule distribution in control (Con)- and shRNA-treated islets (scale bar 5 μm). (B) Insulin granule:plasmamembrane (PM) ratio is unaffected by ADCY5 silencing (n = 18 cells from multiple islets from three donors).
**Supplementary Figure 4:** Epac2-camps expression and localization in human islets. (A) Expression of the cAMP probe *Epac2-camps* is predominantly restricted to beta cells, as shown using immunohistochemistry with antibodies against insulin and glucagon (scale bar, 17.5 µm).
Supplementary Figure 5: Effects of ADCY5-silencing can be mimicked using selective inhibitors of ADCY5 activity. (A) Co-infusion of NKY80 suppresses 11mM glucose (G11)-evoked cytosolic Ca$^{2+}$ rises (left panel; mean traces from a single donor), reducing both AUC and amplitude (right panel) (G3, 3 mM glucose) (**P<0.01 versus Control (Con); Mann-Whitney U-test) ($n = 6$ recordings). (B) ADCY5-silencing does not significantly alter the suppressive effects of NKY80 on AUC and amplitude of Ca$^{2+}$ influx (NS, non-significant; Mann-Whitney U-test) ($n = 4$ recordings). Values represent mean ± SEM.
Supplementary Figure 6: ADCY5 does not alter Ca$^{2+}$ responses to depolarisation. (A) KCl (30 mM) elicits similar responses in control (Con) and shRNA-treated islets (left panel, representative traces) (gray/black, raw; red, smoothed) (G3, 3 mM glucose) (NS, non-significant; Mann-Whitney U-test) ($n$ = 4 recordings). (B) As for (A) but in the continued presence of diazoxide (Dz) 500 µM to limit complications arising from changes in plasma membrane potential ($n$ = 10 recordings). Values represent mean ± SEM.
**Supplementary Table 1: shRNA sequences against ADCY5**

| Clone ID          | Sequence                                                                 |
|-------------------|--------------------------------------------------------------------------|
| TRCN0000078338    | CCGGCGCCATAGACTTCTTCAACAAATCGGTTGAAAGAAGTCTATGGGCTTTTTG                  |
| TRCN0000078339    | CCGGGCGCAGAGAATCACTTCTTACTCTCGAGTAAACAGTATTCTCTGCGGCTTTTTG               |
| TRCN0000078340    | CCGGCTACACTAACTACCTGAATCTCGAGATTGTCAGGTTGTAGTGTAGCTTTTTG                |
| TRCN0000078341    | CCGTCTGTGATCTACTCTCGTGACTCGGAGGAGTAGACAGAGTACACAGAGATTTTTG              |
| TRCN0000078342    | CCGCAACGCATAGACTTCTTCAACACTCGGTTGAAGAAGTCTATGGGCTTTTTG                  |
### Supplementary Table 2: qRT-PCR primer sequences used for SYBR Green assays.

| Gene        | Forward Primer                         | Reverse Primer                         |
|-------------|----------------------------------------|----------------------------------------|
| GLP1R       | 5' ACATCAAATGCAGACTTGCCA 3'            | 5' CCCAGCTTTCCGAAATTCC 3'             |
| ADCY5_human | 5' CAGAAGCGGAAGAAGAAGAAGG 3'           | 5' CCAGAAAATCGATCCACTCCATCC 3'        |
| ADCY5_mouse | 5' GCCAATGCAATAGACTTCAG 3'             | 5' ATCTCTTCTTTCTTTCTTG 3'             |
| ADCY6_human | 5' GGAAACTACAGGCACACAGGG 3'            | 5' GAGGCAAACATAACAGCCAC 3'            |
| ADCY6_mouse | 5' TAAATGCCAGCACCTATGACC 3'            | 5' TGTTCAACCCCGATCTCTGCT 3'           |
| ADCY8_human | 5' CCAATGACCCTCCGTCTC 3'               | 5' GTGAAGACAAAGTACTCTGGG 3'           |
| Cyclophilin(ppia) | 5' AAGACTGAGTGTTGGATGG 3'               | 5' ATGGTGATCTTTCTGGCTG 3'             |
### Supplementary Table 3: qRT-PCR primers for eQTL

| Gene symbol | Gene name                  | Taqman accession       | Location (exon boundary) |
|-------------|----------------------------|-------------------------|--------------------------|
| ADCY5       | Adenylate Cyclase 5        | Hs00766287_m1           | exon 16-17               |
| TBP         | TATA-binding protein       | Hs00427620_m1           | exon 2-3                 |
| Gender | Age (years) | BMI (kg/m²) | Genotype rs11708067 | ADCYS/TBP Expression (2^{ΔCt}) |
|--------|-------------|-------------|---------------------|-------------------------------|
| M      | 47          | 23.50       | AA                  | 1.27                          |
| M      | 39          | 32.60       | AA                  | 1.03                          |
| M      | 67          | 24.20       | AA                  | 1.27                          |
| M      | 58          | 27.80       | AA                  | 0.98                          |
| M      | 59          | 26.73       | AA                  | 0.41                          |
| M      | 53          | 27.77       | AA                  | 2.18                          |
| M      | 61          | 24.80       | AA                  | 1.18                          |
| M      | 22          | 19.60       | AG                  | 1.22                          |
| M      | 56          | 24.70       | AG                  | 1.02                          |
| M      | 33          | 21.80       | AG                  | 2.43                          |
| M      | 66          | 27.77       | AG                  | 3.14                          |
| M      | 51          | 26.23       | AG                  | 1.39                          |
| M      | 52          | 29.98       | AG                  | 2.17                          |
| M      | 59          | 27.68       | AG                  | 3.33                          |

**Supplementary Table 4**: Characteristics of donors used for eQTL analysis.
| Date          | Age (years) | Gender | BMI (kg/m²) | Origin   |
|--------------|-------------|--------|-------------|----------|
| 04 Feb 2014  | 77          | M      | 24.5        | Pisa     |
| 29 Jan 2014  | 40          | M      | 32.9        | Pisa     |
| 8 Jan 2014   | 50          | M      | 36          | Oxford   |
| 20 Dec 2013  | 66          | M      | 28.6        | Geneva   |
| 16 Dec 2013  | 77          | F      | 25.7        | Pisa     |
| 3 Dec 2013   | 50          | F      | 30          | Oxford   |
| 29 Nov 2013  | 47          | M      | 27.5        | Geneva   |
| 23 Nov 2013  | 83          | F      | 27.3        | Pisa     |
| 23 Sep 2013  | 76          | M      | 33.2        | Milan    |
| 16 Sep 2013  | 59          | M      | 25.7        | Milan    |
| 27 Aug 2013  | 20          | M      | unknown     | Alberta  |
| 13 Aug 2013  | 62          | F      | unknown     | Alberta  |
| 27 Jul 2013  | 23          | F      | 22.49       | Pisa     |
| 30 Jun 2013  | 28          | M      | 29          | Geneva   |
| 17 May 2013  | 75          | F      | 19.6        | Pisa     |
| 7 May 2013   | 67          | F      | 22.8        | Milan    |
| 3 May 2013   | 52          | M      | 24.8        | Geneva   |
| 24 Apr 2013  | 28          | F      | 20.2        | Pisa     |
| 19 Mar 2013  | 51          | F      | 41          | Oxford   |
| 28 Feb 2013  | 51          | M      | 30          | Oxford   |
| 27 Feb 2013  | 34          | M      | 31          | Oxford   |
| 11 Jan 2013  | 67          | M      | 27.4        | Geneva   |
| 11 Dec 2012  | 76          | F      | 25.4        | Pisa     |
| 27 Nov 2012  | 68          | M      | 27.5        | Pisa     |
| 12 Nov 2012  | 52          | M      | 27          | Geneva   |
| 29 Oct 2012  | 46          | M      | 36          | Oxford   |

**Supplementary Table 5:** Characteristics of donors used for non-eQTL studies.
SUPPLEMENTARY MOVIE LEGENDS

Supplementary Movie 1: *Timelapse recording of Ca$^{2+}$-responses to 11 mM glucose in a control treated human islet.* A fluo2-loaded islet was recorded at 0.5 Hz for 40 minutes and glucose concentrations elevated at the indicated time point. Playback at 120 frames per second (fps). Movie has been cropped to display a single islet.

Supplementary Movie 2: *Timelapse recording of Ca$^{2+}$-responses to 11 mM glucose in a shRNA-treated human islet.* As for Supplementary Movie 1, but following silencing of ADCY5.

Supplementary Movie 3: *Timelapse recording of Ca$^{2+}$-responses to 20 nM GLP-1 in a control-treated human islet.* As for Supplementary Movie 1, but following application of GLP-1.

Supplementary Movie 4: *Timelapse recording of Ca$^{2+}$-responses to 20 nM GLP-1 in a shRNA-treated human islet.* As for Supplementary Movie 2, but following application of GLP-1.