SUPPLEMENTARY DATA

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).
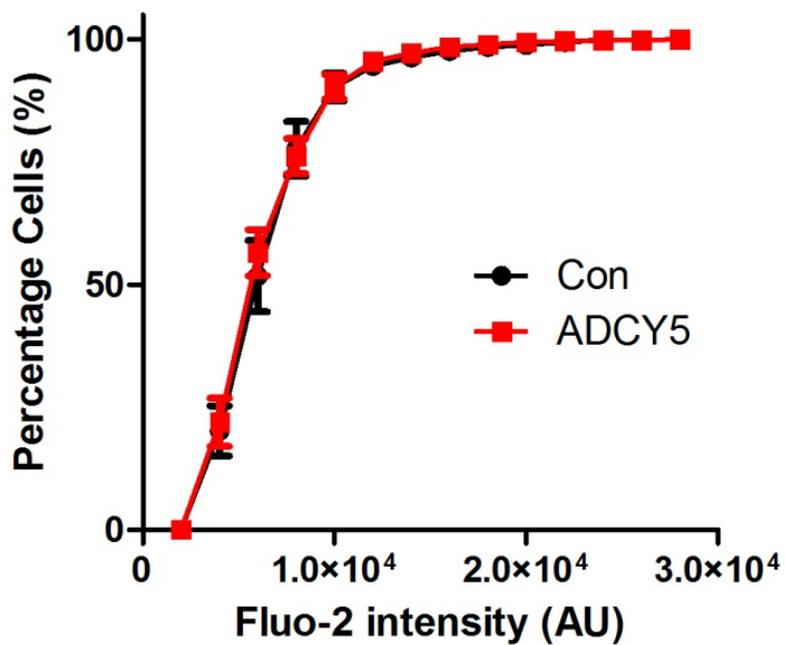
SUPPLEMENTARY DATA

A
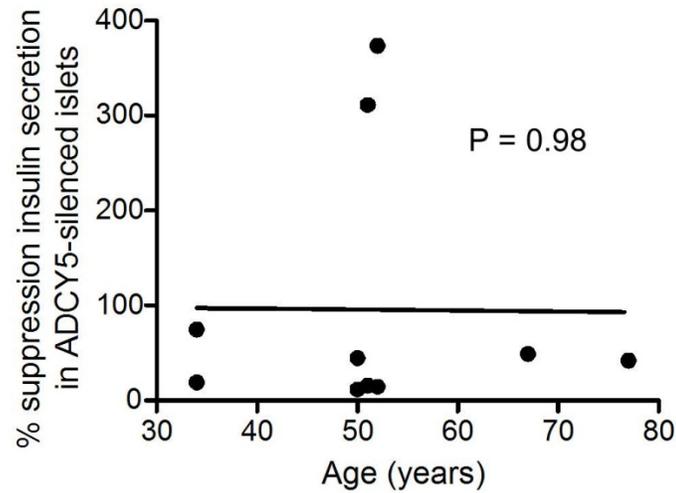
B
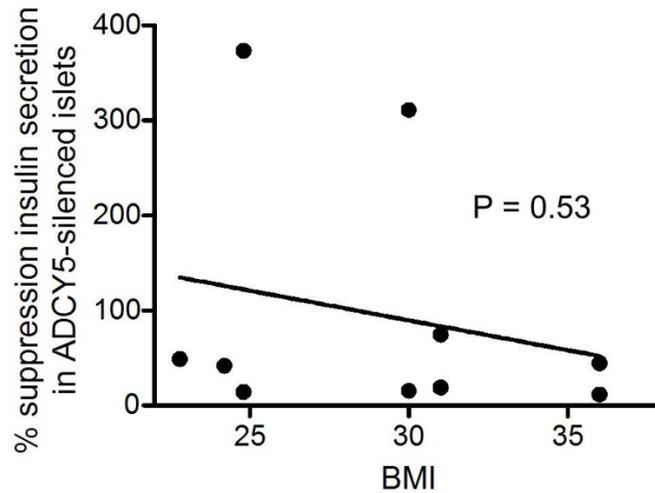
SUPPLEMENTARY DATA

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).
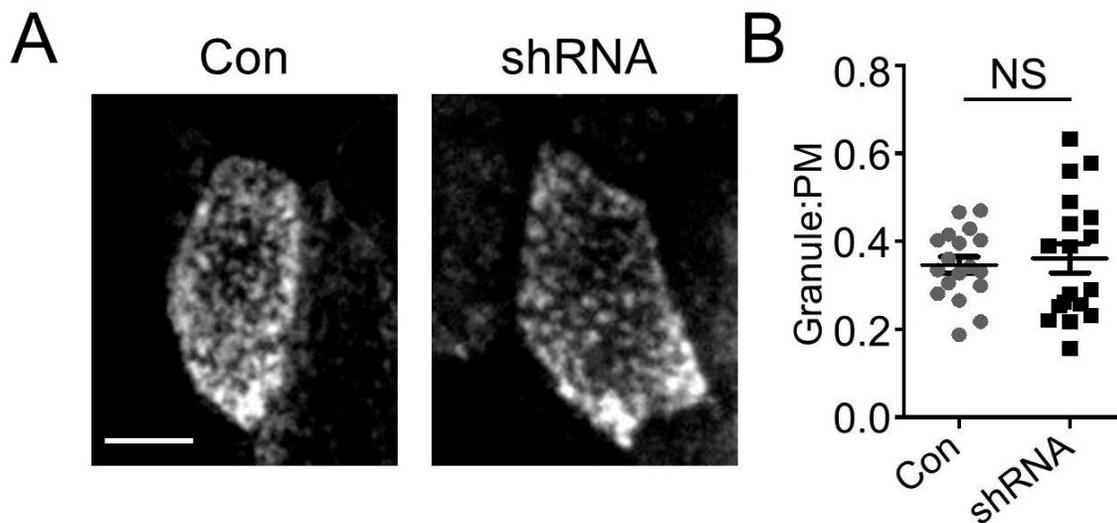
Supplementary Figure 4. *Epac2-camps expression and localization in human islets.* **(A)** Expression of the cAMP probe *Epac2-camps* is predominantly restricted to beta cells, as shown using immunohistochemistry with antibodies against insulin and glucagon (scale bar, 17.5 µm).
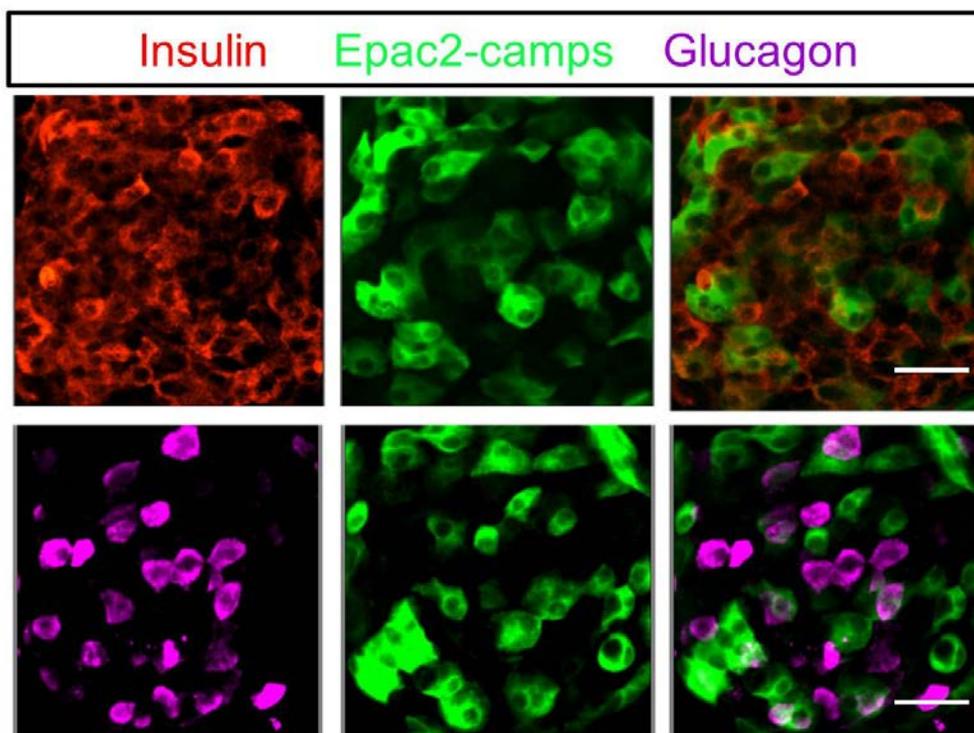
SUPPLEMENTARY DATA

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.
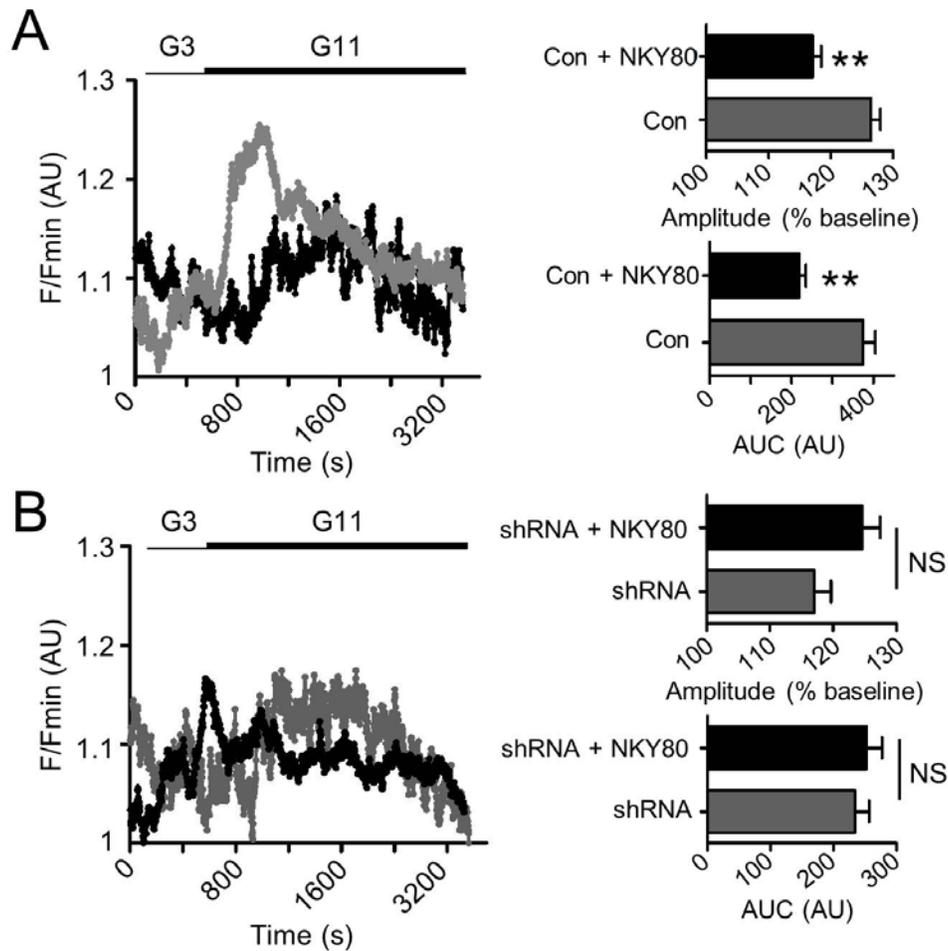
Supplementary Figure 6. *ADCY5 does not alter Ca^{2+} responses to depolarisation.* **(A)** KCl (30 mM) elicits similar responses in control (Con) and shRNA-treated islets (left panel, representative traces) (gray/black, raw; red, smoothed) (G3, 3 mM glucose) (NS, non-significant; Mann-Whitney U-test) (*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.
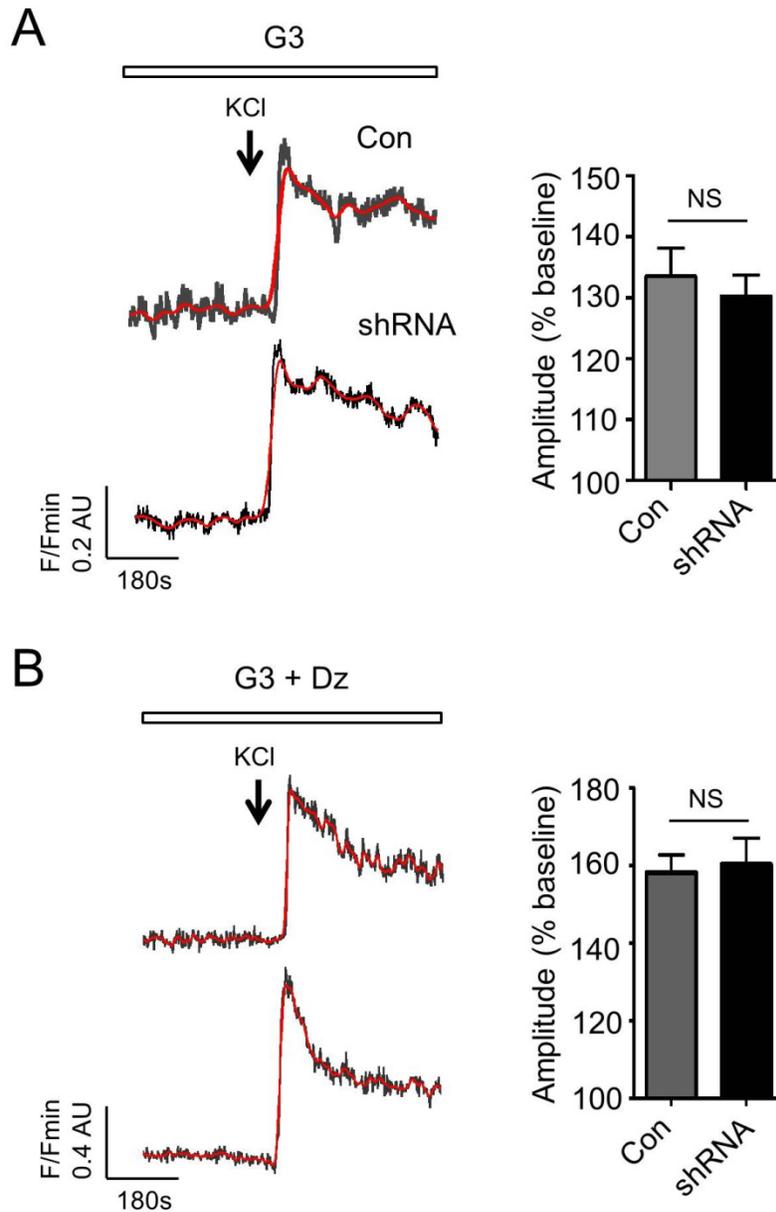
SUPPLEMENTARY DATA

Supplementary Table 1. *shRNA sequences against ADCY5.*

Clone ID	Sequence
TRCN0000078338	CCGGCGCCATAGACTTCTTCAACAACTCGAGTTGTTGAAGAAGTCTATGGCGTTTTTG
TRCN0000078339	CCGGGCCGCAGAGAATCACTGTTTACTCGAGTAAACAGTGATTCTCTGCGGCTTTTTG
TRCN0000078340	CCGGGCTACACTCAACTACCTGAATCTCGAGATTCAGGTAGTTGAGTGTAGCTTTTTG
TRCN0000078341	CCGGTCTGTGATCTACTCCTGCGTACTCGAGTACGCAGGAGTAGATCACAGATTTTTG
TRCN0000078342	CCGGCAACGCCATAGACTTCTTCAACTCGAGTTGAAGAAGTCTATGGCGTTGTTTTTG

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

Gene	Forward Primer	Reverse Primer
GLP1R	5' ACATCAAATGCAGACTTGCCA 3'	5' CCCAGCTCTTCCGAAATTCC 3'
ADCY5_human	5' CAGAAGCGGAAAGAAGAGAAGG 3'	5' CCAGAAACTCATCCACTTCATCC 3'
ADCY5_mouse	5' GCCAATGCCATAGACTTCAG 3'	5' ATCTCCTCCTTCTCTTCTGTG 3'
ADCY6_human	5' GGAAACTACAGGCAACAGGG 3'	5' GAGGCAAACATAACAGCCAC 3'
ADCY6_mouse	5' TAAATGCCAGCACCTATGACC 3'	5' TGTTCAACCCGATCTTCATCTG 3'
ADCY8_human	5' CCAATGACCATCCAGTTCTC 3'	5' GTGAAGACAAAGTACTCTGGG 3'
Cyclophilin(ppia)	5' AAGACTGAGTGGTTGGATGG 3'	5' ATGGTGATCTTCTTGCTGGT 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

SUPPLEMENTARY DATA

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

Gender	Age (years)	BMI (kg/m^2)	Genotype rs11708067	*ADCY5/TBP* Expression ($2^{-\Delta C_T}$)
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