SUPPLEMENTARY DATA

Supplementary Table 1. Information on islet donors

Center	Diabetes status	n	Median age in years (range)	Median BMI in kg/m^2 (range)
Lund University	Non-diabetic	56	57 (27-73)	25 (18-34)
	Diabetic	20	60 (41-75)	27 (23-37)
University of Oxford	Non-diabetic	16	56 (24-73)	31 (23-37)
	Diabetic	8	59 (34-75)	27 (22-44)

Supplementary Table 2. Phenotype data from donors

Number of phenotypes/functional parameters measured from each donor	Number of donors (total: 100)
1	34
2	19
3	35
4	8
5	4

Human islets from a total of 100 donors were collected. Insulin secretion, insulin content, ATP content, membrane capacitance and granule distribution were measured in 59, 53, 56, 36 and 18 different preparations, respectively. It was possible to measure all five parameters for only 4 preparations and only 1 parameter for 34 of the preparations. DNA was available from all donors.

Supplementary Table 3. SNPs analyzed in the study.

SNP	Nearest gene	Risk allele	Non-risk allele	Risk allele frequency
rs7903146	*TCF7L2*	T	C	0.24
rs2237895	*KCNQ1*	C	A	0.5
rs231362	*KCNQ1*	G	A	0.47
rs13266634	*SLC30A8*	T	C	0.3
rs5219	*KCNJ11*	T	C	0.43
rs10830963	*MTNR1B*	G	C	0.39
rs553668	*ADRA2A*	A	G	0.12
rs1111875	*HHEX/IDE*	C	T	0.51
rs10946398	*CDKAL1*	C	G	0.39
rs10423928	*GIPR*	C	A	0.27
rs2191349	*DGKB*	G	T	0.22
rs10861975	*SYT1*	T	C	0.25
rs363004	*SNAP25*	A	G	0.12
rs11759297	*RIMS1*	G	A	0.17
rs560887	*G6PC2*	T	C	0.35
rs11920090	*SLC2A2*	A	T	0.22
rs4607517	*GCK*	A	G	0.2

The nearest genes, risk/non-risk alleles for type-2 diabetes or impaired glucose tolerance, and risk allele frequency in the donors are given for each SNP.

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Supplementary Table 4. Genotype effects on β-cell exocytosis

Entire cohort (n=249 cells from 36 donors)							
SNP	Exocytosis*				Charge†	Exocytosis vs. Charge‡	
	P value	Effect§	Lower‖	Upper‖	P value	P value	Effect
rs7903146 (*TCF7L2*)	0.007	-6.40	-11.1	-1.77	0.47	0.127	-1.0
rs553668 (*ADRA2A*)	0.059	-15.4	-31.7	0.58	0.43	0.201	-11.0
rs5219 (*KCNJ11*)	0.021	-7.00	-13.0	-1.04	0.18	0.062	-3.0
rs2237895 (*KCNQ1*)	0.016	-10.1	-18.2	-1.86	0.23	0.32	-0.32
Donors with BMI<31 kg/m^2 (n=170 cells from 24 donors)							
SNP	Exocytosis				Charge	Exocytosis vs. Charge	
	P value	Effect	Lower	Upper	P value	P value	Effect
rs7903146 (*TCF7L2*)	0.004	-8.4	-14.1	-2.6	0.81	0.002	-13.0
rs553668 (*ADRA2A*)	1.50E-06	-23.6	-33.2	-14.0	0.22	3.00E-05	-24.9
rs5219 (*KCNJ11*)	0.03	-7.8	-14.9	-0.76	0.48	0.004	-5.1
rs2237895 (*KCNQ1*)	0.001	-10.4	-16.8	-4.0	0.49	0.636	-2.0

* Exocytosis measured as the total increase in single β-cell capacitance in response to ten depolarizations
† The maximal integrated Ca^{2+}-current evoked by a 50 ms depolarization during a current-voltage protocol.
‡ The capacitance increase in response to the first depolarization normalized to the charge, indicating the Ca^{2+} sensitivity of exocytosis
§ Effect of each additional risk allele on exocytosis (fF/pF), charge (pC) or exocytosis vs. charge (fF/pC) estimated from the linear model.
‖ Lower and upper limits of a 95% confidence interval for the effect.

Supplementary Table 5. Genotype effect on granule distribution measured as Ns*

SNP	P value	Effect‡	Lower§	Upper
rs7903146† (*TCF7L2*)	0.40	-0.0040	-0.13	0.054
rs553668 (*ADRA2A*)	0.03	-0.14	-0.26	-0.018
rs5219 (*KCNJ11*)	0.05	-0.12	-0.25	-0.001
rs2237895 (*KCNQ1*)	0.04	-0.10	-0.19	-0.006

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* Ns is determined from the electron micrographs of human islets as the number of granules located within 150 nm from the plasma membrane divided by cell perimeter divided by granule diameter. Islets were incubated at high glucose for 1 h prior to fixation for electron microscopy.
† n=97 cells from 18 donors
‡ Effect of each additional risk allele on granule distribution (Ns: granules/μm^2) estimated from the linear model.
§ Lower and upper limits of a 95% confidence interval for the effect.

Supplementary Table 6. Gene expression analysis

		ADRA2A	*KCNJ11*	*KCNQ1*	*TCF7L2* full	*TCF7L2* isoform
Non-diabetic donors (n=56)	Mean	1.93	0.531	0.412	0.94	0.45
	s.e.m.	0.19	0.030	0.055	0.13	0.054
Donors with T2D (n=15)	Mean	1.37	0.379	0.376	0.86	0.38
	s.e.m.	0.16	0.048	0.100	0.16	0.071
Summary	Ratio T2D vs. ND	0.71	0.71	0.91	0.92	0.84
	p value	0.15	0.022	0.75	0.80	0.52

Gene expression in human islets. Islets were homogenized in Qiazol reagent (Qiagen, USA) followed by vortexing. RNA was extracted with chloroform precipitation using the mRNeasy kit (Qiagen). Gene expression was measured by qPCR using TaqMan (Applied Biosystems). For each condition and gene, the expression was measured from triplicate wells using Applied Biosystems 7900HT RT-PCR system and normalized to the geometric mean of *HPRT* and *UBC*. Inventoried TaqMan assays were used for all genes, except *TCF7L2*, where custom assays were used to detect the full and the truncated transcript, respectively, as described in (Locke et al. Diabetologia 2011)). Shown are mean and s.e.m. for the expression of each gene in non-diabetic and T2D donors. P values using Student's *t*-test.

Supplementary Table 7. Genotype effects on islet phenotypes in non-diabetic donors

		Insulin secretion (n=33 donors)		Total exocytosis* (n=189 cells from 28 donors)		Ns† (n=60 cells from 12 donors)	
SNP	Nearest gene	p value	Effect‡	p value	Effect	p value	Effect
rs7903146	*TCF7L2*	0.071	-0.488	0.140	-2.21	0.334	-0.031
rs553668	*ADRA2A*	0.478	-0.293	0.712	-11.4	0.059	-0.202
rs5219	*KCNJ11*	0.338	-0.138	0.015	-8.17	0.052	-0.167
rs2237895	*KCNQ1*	0.142	-0.249	0.047	-10.1	0.249	-0.107

* Exocytosis measured as the total increase in single β-cell capacitance in response to ten depolarizations
† Number of docked insulin granules
‡ Effect of each additional risk allele on insulin secretion (ng/islet/h), exocytosis (fF/pF), or granule distribution (Ns: granules/μm^2) estimated from the linear model.

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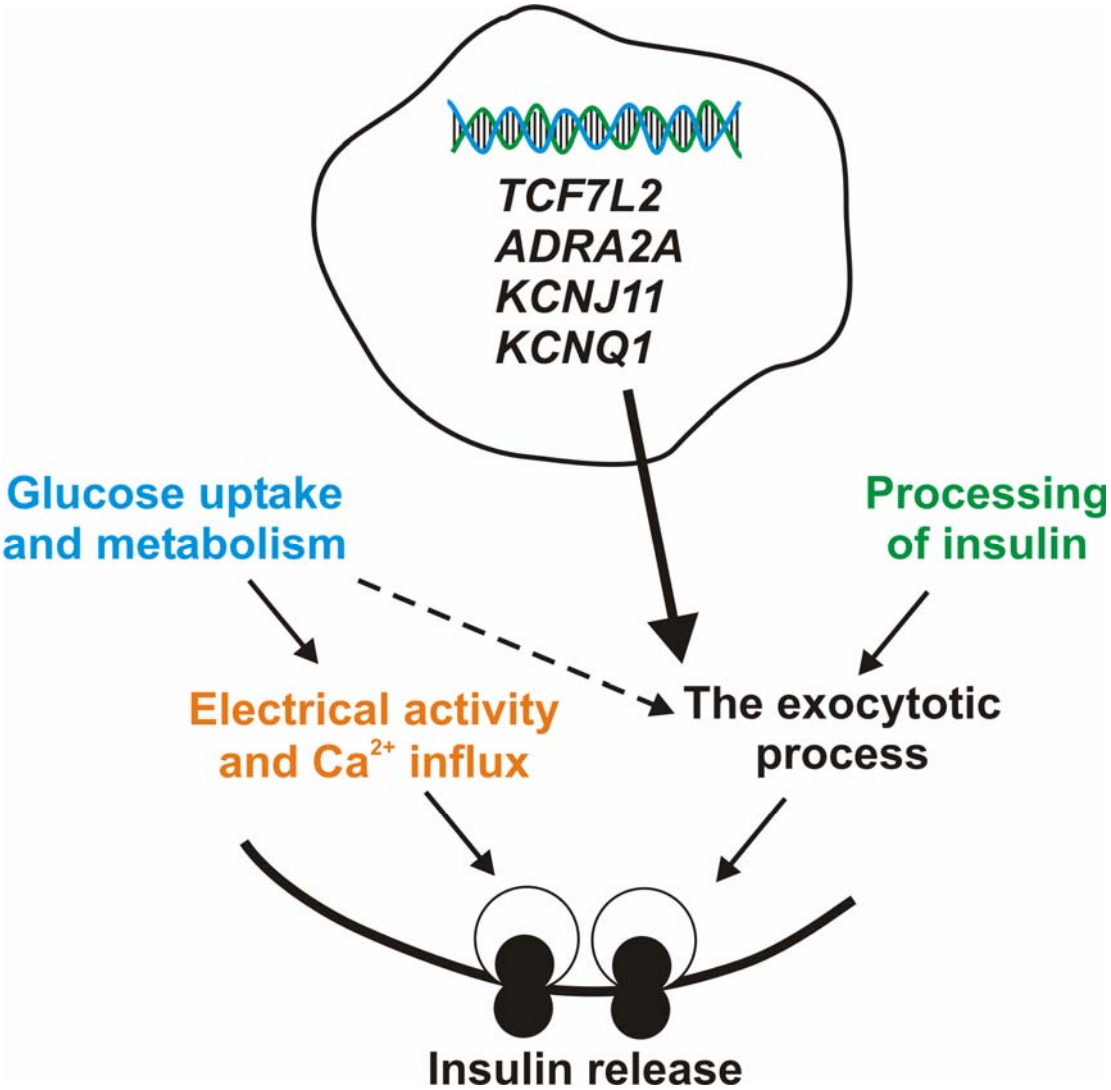
Supplementary Table 8. Samples used in this study

Cohort	N (with diabetes)	Geographic origin	Age (y)	BMI (kg/m^2)
Botnia IVGTT	604	Finland	45 ± 13	25.5 ± 4.1
Botnia prospective cohort	2770 (138)	Finland	45 ± 14	25.6 ± 4.1

Data are shown as mean ± s.d.

Supplementary Figure 1. Insulin secretion is depending on: 1) the activation of the stimulus-secretion coupling including glucose uptake and metabolism, resulting in increased cytosolic ATP, initiation of electrical activity and acceleration of Ca^{2+}-dependent exocytosis; and 2) the processing of insulin granules and the exocytotic process. The exocytotic machinery is fine-tuned via several processes, including those that determine the Ca^{2+} sensitivity of exocytosis and docking of the granules to the plasma membrane. We demonstrate that genetic risk variants near *TCF7L2*, *ADRA2A*, *KCNJ11* and *KQNQ1* contribute to reduced insulin secretion via a defective exocytotic process.



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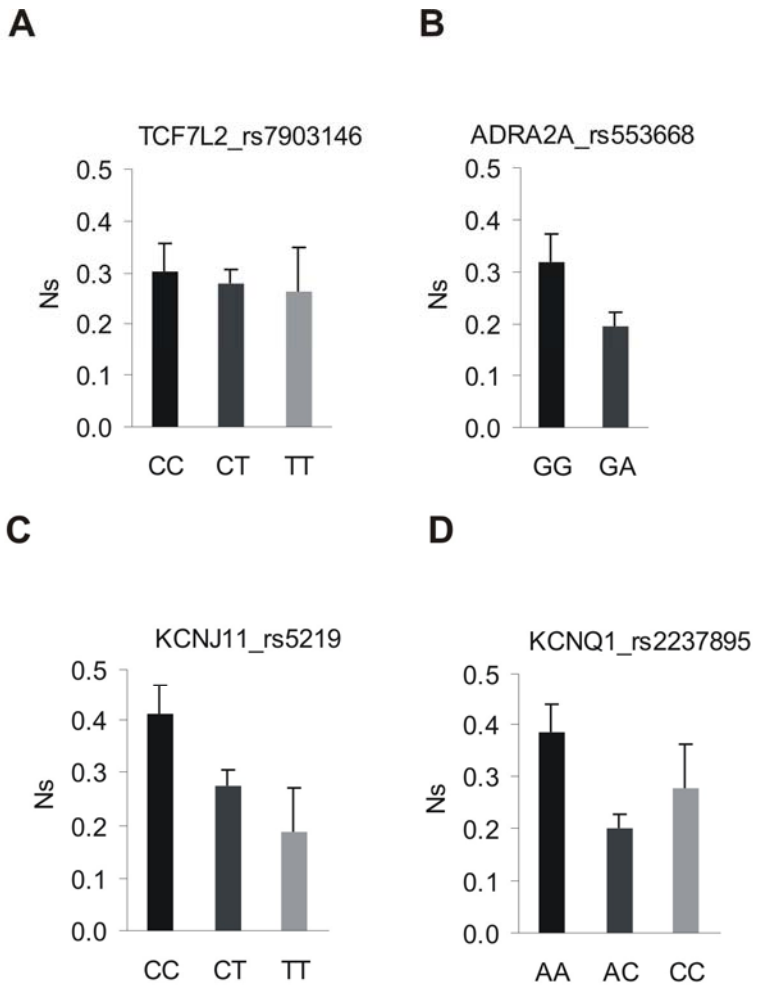
Supplementary Figure 2. A-D: The histograms show the number of docked granules as surface-density (Ns; granules/μm^2) in β-cells from different genotype carriers. Data are presented as means ± s.e.m. Statistical analyses for each variant were done using a linear model and are presented in Table 1 and Supplementary Table 5. Number of cells analyzed are in A: 33 CC, 60 CT and 4 TT; B: 72 GG and 25 GA; C: 18 CC, 71 CT and 8 TT; D: 40 AA, 40 AC and 17 CC.



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Supplementary Figure 3. *KCNQ1* silencing. A: Immunostaining of a β-cell from human islets treated with a negative control siRNA. Green shows staining using an anti-insulin antibody from guinea-pig (Linco) at 1:500 dilution. Red shows staining with an anti-KCNQ1 antibody from rabbit (Alomone Labs) at 1:100 dilution. Scale bar is 5 μm. Bottom right shows overlay.
B: As in A but immunostaining of a β-cell from human islets treated with siRNA targeting *KCNQ1*.
C: Histogram shows average fluorescence of anti-KCNQ1 antibody in β-cells from islets treated with control siRNA or siRNA against *KCNQ1* (n=8 cells per group). *P<0.05.
D: Immunoblots of total protein from human islets using polyclonal KCNQ1 antisera. KCNQ1 was reduced by 54% in islets treated with siRNA against KCNQ1 compared with islets treated with control siRNA. Data were normalized to actin.

For the Western blots human islets were transferred to a homogenization buffer, sonicated and frozen. The homogenization buffer consisted of 250 mM sucrose, 5 mM HEPES, 0.5 mM EGTA (pH 7.4 with KOH) and complete protease inhibitor cocktail (Roche). Thirty micrograms of total protein was electrophoresed on 10% SDS-PAGE, and the separated proteins were transferred onto a polyvinylidine fluoride membrane (Amersham Pharmacia Biotech). The membrane was blocked with 5% w/v nonfat dry milk in TBS with 0.1% Tween for 1 h at room temperature, followed by incubation with the primary antibodies against KCNQ1 (1:200) or actin (1:500). After washing, the membrane was incubated with peroxidase-linked anti-rabbit IgG (1:25000) (Amersham) overnight at 4°C. Signal was detected by a chemiluminescence kit (Thermo Scientific).