Loss of ZnT8 function protects against diabetes by enhanced insulin secretion

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Supplementary Tables (1-8)

Supplementary Table 1: Clinical characteristics of carriers of the p.Arg138* and p.Trp325Arg variants in *SLC30A8* participating in the test meal.

Measurements	p.Arg138*	p.Arg138Arg	*p	p.Trp325Trp	p.Arg325	#p
	Mean (SEM)	Mean (SEM)		Mean (SEM)	Mean (SEM)	
Numbers (M/F)	54 (29/25)	47 (24/23)		16 (10/6)	31 (14/17)	
Age (years)	50.74 (2.09)	53.39 (2.17)	0.961	52.75 (3.68)	53.73 (2.72)	0.779
BMI (kg/m^2)	27.41 (0.59)	26.12 (0.43)	**0.047**	27.14 (0.73)	25.59 (0.51)	0.726
Glucose(mmol/L)	5.36 (0.09)	5.43 (0.1)	0.186	5.41 (0.09)	5.45 (0.14)	0.377
HbA1C (%)	5.34 (0.06)	5.43 (0.06)	0.108	5.45 (0.14)	5.42 (0.07)	0.833
Cholesterol (mmol/L)	5.21 (0.19)	5.46 (0.17)	0.234	6.03 (0.3)	5.03 (0.15)	**0.005**
HDL-cholesterol (mmol/L)	1.38 (0.07)	1.41 (0.05)	0.633	1.38 (0.11)	1.43 (0.05)	0.977
LDL-cholesterol (mmol/L)	3.26 (0.17)	3.54 (0.15)	0.175	4.05 (0.26)	3.14 (0.14)	**0.012**
Triglycerides (mmol/L)	1.25 (0.09)	1.13 (0.07)	0.784	1.28 (0.13)	1 (0.08)	0.095

M; Male, F; female. * p values from family-based association (QTDT[1]) after 100,000 permutations. # p values calculated using linear regression. Bolded p values are <0.05.

Supplementary Table 2: Clinical characteristics of carriers of the SLC30A8-p.Arg138* participating in the oral glucose tolerance test (OGTT) in family studies.

Measurements	OGTT-test meal families					OGTT- families				
	p.Arg138*		p.Arg138Arg			p.Arg138*		p.Arg138Arg		
	N	Mean (SEM)	N	Mean (SEM)	p	N	Mean (SEM)	N	Mean (SEM)	p
Genotype (M/F)	31 (19/12)		13 (5/8)			20 (9/11)		240 (124/116)		
Age (years)	31	49.6 (2.85)	13	53.19 (4.23)	0.68	20	41.2 (3.74)	240	38.6 (0.91)	0.41
BMI (kg/m^2)	31	27.07 (0.66)	13	26.48 (0.77)	0.60	20	25.57 (0.76)	240	24.74 (0.25)	0.42
Cholesterol (mmol/L)	31	5.09 (0.29)	13	5.62 (0.32)	0.46	20	4.81 (0.21)	240	4.99 (0.06)	0.36
HDL-cholesterol (mmol/L)	31	1.29 (0.08)	13	1.42 (0.09)	0.59	20	1.23 (0.06)	239	1.34 (0.02)	0.07
LDL-cholesterol (mmol/L)	31	3.45 (0.28)	13	3.9 (0.28)	0.30	10	2.86 (0.25)	165	3.2 (0.07)	0.22
Triglycerides (mmol/L)	31	1.27 (0.12)	13	1.24 (0.13)	0.55	20	1.03 (0.09)	240	1.05 (0.03)	0.59
Fasting glucose (mmol/L)	31	5.12 (0.11)	13	5.15 (0.19)	0.206	20	5.29 (0.11)	240	5.73 (0.03)	**0.001**
Glucose at 30 min. (mmol/L)	31	7.86 (0.38)	13	7.79 (0.63)	0.973	20	8.31 (0.35)	240	8.33 (0.10)	0.770
Glucose at 120 min. (mmol/L)	31	6.03 (0.41)	13	7.2 (1.21)	0.966	20	6.79 (0.44)	240	6.18 (0.09)	0.226
Fasting insulin (mU/L)	31	10.37 (1.03)	13	11.26 (1.47)	0.204	19	6.66 (0.77)	240	6.06 (0.32)	0.460
Insulin at 30 min. (mU/L)	31	99.78 (14.36)	13	65.98 (8.65)	0.165	20	72.94 (13.37)	238	43.71 (1.98)	**0.041**
Insulin at 120 min. (mU/L)	31	71.35 (12.69)	13	76.1 (16.52)	0.946	19	71.08 (22.17)	238	31.17 (1.86)	0.111

N; Numbers, M; male, F; female, OGTT- test meal families; test meal families members that took part in OGTT during their second visit; OGTT- families; a group of p.Arg138* carriers (N=20, 8 families) and their family members with OGTT data. A mixed model adjusting for genetic relationships was used to calculate p values. Bolded p values are < 0.05.

Supplementary Table 3: Clinical characteristics of carriers of the p.Arg138* and p.Trp325Arg variants in *SLC30A8* participating in the population based oral glucose tolerance test (OGTT-population).

Measurements	p.Arg138*		p.Arg138Arg			p.Trp325Trp		p.Arg325		
	N	Mean (SEM)	N	Mean (SEM)	P	N	Mean (SEM)	N	Mean (SEM)	P
Genotype (M/F)	35 (19/16)		8141 (3747/4394)			1248 (577/671)		6893 (3170/3723)		
Age (years)	35	44.28 (2.56)	8141	47.75 (0.17)	0.207	1248	48.3 (0.43)	6893	47.65 (0.19)	0.718
BMI (kg/m^2)	35	26.95 (0.79)	8129	26.01 (0.05)	0.175	1246	26.2 (0.12)	6883	25.98 (0.05)	0.217
Cholesterol (mmol/L)	35	5.1 (0.18)	8059	5.39 (0.01)	0.225	1239	5.41 (0.03)	6820	5.39 (0.01)	0.673
HDL-cholesterol (mmol/L)	35	1.38 (0.07)	8035	1.4 (0)	0.884	1237	1.41 (0.01)	6798	1.4 (0)	0.277
LDL-cholesterol (mmol/L)	35	3.17 (0.16)	5798	3.3 (0.01)	0.531	894	3.33 (0.03)	4904	3.3 (0.01)	0.981
Triglycerides (mmol/L)	35	1.21 (0.11)	8059	1.29 (0.01)	0.356	1239	1.27 (0.02)	6820	1.29 (0.01)	0.676

N; Numbers, M; male, F; female. All p values calculated by linear regression.

Supplementary Table 4: Association of p.Arg138* and p.Trp325Arg variants in *SLC30A8* with measures of insulin secretion during oral glucose tolerance test (OGTT) and intravenous glucose tolerance test (IVGTT).

	()	p.Arg138*		p.Arg138Arg			p.Arg138*	p.Arg325			p.Trp325Trp		p.Arg325		
OGTT	Time (min)	N	Mean (SEM)	N	Mean (SEM)	*p	N	Mean (SEM	N	Mean (SEM	#p	N	Mean (SEM	N	Mean (SEM	*p
Glucose (mmol/L)	0	35	5.11 (0.1)	8139	5.36 (0.01)	**0.033**	35	5.11 (0.1)	6891	5.36 (0.01)	**0.025**	1248	5.34 (0.02)	6891	5.36 (0.01)	0.353
Glucose (mmol/L)	30	35	7.91 (0.25)	8141	8.33 (0.02)	0.145	35	7.91 (0.25)	6893	8.34 (0.02)	0.141	1248	8.25 (0.04)	6893	8.34 (0.02)	0.224
Glucose (mmol/L)	120	35	5.04 (0.2)	8129	5.61 (0.02)	0.132	35	5.04 (0.2)	6883	5.63 (0.02)	0.139	1246	5.53 (0.04)	6883	5.63 (0.02)	0.162
Insulin (mU/L)	0	35	8.51 (0.74)	8084	7.49 (0.07)	0.118	35	8.51 (0.74)	6844	7.51 (0.08)	0.132	1240	7.38 (0.15)	6844	7.51 (0.08)	0.515
Insulin (mU/L)	30	35	88.75 (10.87)	7980	64.45 (0.51)	**0.010**	35	88.75 (10.87)	6746	64.12 (0.56)	**9.9×10^{-3}**	1234	66.25 (1.29)	6746	64.12 (0.56)	**2.9×10^{-3}**
Insulin (mU/L)	120	34	36.82 (4.33)	7959	40.01 (0.48)	0.639	34	36.82 (4.33)	6733	40.24 (0.52)	0.672	1226	38.78 (1.16)	6733	40.24 (0.52)	0.552
Insulin/Glucose	0	35	1.7 (0.16)	8082	1.4 (0.01)	**0.048**	35	1.7 (0.16)	6842	1.41 (0.01)	0.053	1240	1.39 (0.03)	6842	1.41 (0.01)	0.423
Insulin/Glucose	30	35	11.63 (1.41)	7980	7.86 (0.06)	**1.9×10^{-3}**	35	11.63 (1.41)	6746	7.81 (0.07)	**1.9×10^{-3}**	1234	8.13 (0.15)	6746	7.81 (0.07)	**1.2×10^{-3}**
Insulin/Glucose	120	34	7.1 (0.72)	7954	6.71 (0.06)	0.278	34	7.1 (0.72)	6728	6.73 (0.07)	0.306	1226	6.62 (0.16)	6728	6.73 (0.07)	0.949
Incremental Insulin	0-30	35	80.24 (10.4)	7980	56.98 (0.48)	**7.6×10^{-3}**	35	80.24 (10.4)	6746	56.65 (0.53)	**7.1×10^{-3}**	1234	58.83 (1.23)	6746	56.65 (0.53)	**1.5×10^{-3}**
Proinsulin (pmol/L)	0	34	‡9.89 (0.54)	4414	‡11.19 (0.09)	‡0.129	34	9.89 (0.54)	3705	11.26 (0.1)	0.094	709	10.83 (0.24)	3705	11.26 (0.1)	**6.4×10^{-3}**
Proinsulin (pmol/L)	120	--	--	--	--	--	--	--	--	--	--	695	35.96 (0.82)	3664	37.8 (0.34)	**0.039**
IVGTT																
Incremental Insulin/Glucose	0-10	--	--	--	--	--	--	--	--	--	--	86	1.97 (0.17)	458	1.58 (0.06)	**2.6×10^{-3}**

Data are Mean ± SEM, N; Numbers, A star (*) indicates significance for additive effects, calculated using mixed model and adjusting for age, sex, BMI and genetic relatedness. A hash sign (#) indicates significance for additive effects, calculated using mixed model and adjusting for age, sex, BMI, genetic relatedness and status of p.Arg325Trp - genotype. All quantitative traits were inversely normally transformed before association analyses (see Methods). Note (‡) that the association of fasting proinsulin with p.Arg138* for approximately similar samples size has been also reported previously[2]. Bolded p values are < 0.05.

Supplementary Table 5: Meta-analysis of the variant *SLC30A8*-p.Arg138* with oral glucose tolerance test (OGTT) indices from different OGTT cohorts.

		OGTT-test meal families			OGTT- families			OGTT-population			Meta-analysis		
OGTT indices	Time (min)	N	Beta (SE)	p	N	Beta (SE)	p	N	Beta (SE)	p	N	Beta (SE)	p
Glucose	0	44 (31)	-0.32 (0.26)	0.206	260 (20)	-0.74 (0.23)	**0.001**	8174 (35)	-0.35 (0.16)	**0.033**	8478 (86)	-0.45 (0.12)	**1.4×10^{-4}**
Glucose	30	44 (31)	0.01 (0.39)	0.973	260 (20)	-0.07 (0.22)	0.770	8176 (35)	-0.23 (0.16)	0.145	8480 (86)	-0.16 (0.12)	0.202
Glucose	120	44 (31)	-0.01 (0.29)	0.966	260 (20)	0.25 (0.21)	0.226	8164 (35)	-0.24 (0.16)	0.132	8468 (86)	-0.05 (0.12)	0.657
Insulin	0	44 (31)	-0.35 (0.27)	0.204	259 (19)	0.18 (0.24)	0.460	8119 (35)	0.23 (0.15)	0.118	8422 (85)	0.12 (0.11)	0.305
Insulin	30	44 (31)	0.49 (0.36)	0.165	258 (20)	0.50 (0.24)	**0.041**	8015 (35)	0.41 (0.16)	**0.010**	8317 (86)	0.44 (0.12)	**3.8×10^{-4}**
Insulin	120	44 (31)	0.02 (0.25)	0.946	257 (19)	0.37 (0.24)	0.111	7993 (34)	0.07 (0.16)	0.639	8294 (84)	0.13 (0.12)	0.245
Incremental Insulin	0-30	44 (31)	0.65 (0.38)	0.085	257 (19)	0.47 (0.25)	0.055	8015 (35)	0.43 (0.16)	**7.6×10^{-3}**	8316 (85)	0.46 (0.13)	**2.4×10^{-4}**
CIR	30	44 (31)	0.30 (0.44)	0.495	258 (20)	0.41 (0.24)	0.089	8015 (35)	0.41 (0.16)	**0.013**	8317 (86)	0.40 (0.13)	**0.002**

N; Total numbers of individuals (p.Arg138* carriers), OGTT; oral glucose tolerance test, OGTT- test meal families; test meal families members that participated in a OGTT during their second visit, OGTT- families; a group of p.Arg138* carriers (N=20, 8 multiplex families) and their family members, OGTT-population; population based collection of p.Arg138* carriers and non-carriers from Botnia region. Beta (SE); p.Arg138* effect size and standard error (standardized units) calculated from inverse normally transformed data using mixed model adjusting for age, sex, BMI and genetic relatedness using GCTA[3] (see Methods). Meta-analysis performed using fixed effect model as implemented in METAL[4]. Bolded p values are < 0.05.

Supplementary Table 6: Association of p.Arg138* and p.Trp325Arg variants in *SLC30A8* gene with measures of insulin secretion and AUCs during test meal.

	Time (min)	p.Arg138*	p.Arg138Arg	*p	p.Arg138*	p.Arg325	*p	p.Trp325Trp	p.Arg325	#p
Number		54	47		54	31		16	31	
CIR	0-20	442.9 (52.75)	285.16 (27.35)	**0.046**	442.91 (52.75)	242.88 (23.92)	**3.9×10^{-3}**	367.07 (62.01	242.88 (23.92)	0.236
Incremental Insulin	0-20	68.19 (8.36)	54.47 (5.38)	0.577	68.19 (8.36)	41.9 (5.12)	**9.7×10^{-3}**	78.81 (9.97)	41.9 (5.12)	**0.033**
Incremental Insulin/Glucose	0-20	10.06 (1.21)	7.58 (0.73)	0.381	10.06 (1.21)	5.96 (0.67)	**6.0×10^{-3}**	10.73 (1.45)	5.96 (0.67)	**0.044**
Incremental C-peptide	0-20	1.16 (0.1)	1.02 (0.08)	0.617	1.16 (0.1)	0.82 (0.08)	**0.018**	1.39 (0.16)	0.82 (0.08)	**0.023**
AUC Glucose	0-40	253.67 (4.94)	264.87 (5.24)	**0.020**	253.67 (4.94)	263.74 (7.29)	0.060	267.06 (6.39)	263.74 (7.29)	0.201
AUC Insulin	0-40	2549.47 (246.97)	2148.55 (161.66)	0.446	2549.47 (246.97)	1784.99 (168.24)	**0.026**	2852.96 (274.91)	1784.99 (168.24)	**0.022**
AUC Insulin/Glucose	0-40	10.33 (1.03)	8.21 (0.62)	0.192	10.33 (1.03)	6.85 (0.62)	**3.8×10^{-3}**	10.85 (1.14)	6.85 (0.62)	**0.049**
AUC C-peptide	0-40	71.15 (3.37)	63.61 (2.92)	0.476	71.15 (3.37)	58.18 (3.43)	0.061	74.14 (4.46)	58.18 (3.43)	0.054
AUC Glucose	0-190	1045.39 (30.64)	1104.14 (43.2)	0.127	1045.39 (30.64)	1122.42 (58.2)	0.069	1068.72 (59.69)	1122.42 (58.2)	0.927
AUC Insulin	0-190	10128.55 (1003.78)	9013.98 (631.85)	0.928	10128.55 (1003.78)	8689.05 (832)	0.445	9623.23 (946.64)	8689.05 (832)	0.292
AUC Insulin/Glucose	0-190	9.55 (0.83)	8.27 (0.51)	0.711	9.55 (0.83)	7.75 (0.61)	0.317	9.25 (0.86)	7.75 (0.61)	0.363
AUC C-peptide	0-190	376.39 (18.66)	353.91 (15.54)	0.856	376.39 (18.66)	359.47 (21.43)	0.977	343.49 (20.18)	359.47 (21.43)	0.996

Data are Mean ± SEM, AUC; area under curve. A star (*) indicates significance for family-based association (using QTDT[1]) after 100,000 permutations, adjusted for age, sex and BMI for left panel and age, sex, BMI and genotype of p.Trp325Arg for middle panel. A hash sign (#) indicates significance calculated using family-based QFAM test using 100,000 permutations as implemented in PLINK[5] (see Method). Bolded p values are < 0.05.

Supplementary Table 7: Clinical and metabolic features in relation to carrier status of p.Trp325Arg in patients with newly diagnosed type 2 diabetes from Verona Newly Diagnosed Diabetes Study.

Measurements	p.Trp325Trp		p.Arg325Trp		p.Arg325Arg		p Value (by ANOVA)
	N	Mean (SEM)	N	Mean (SEM)	N	Mean (SEM)	
Age (years)	37	59.0 (1.50)	241	57.2 (0.67)	310	59.0 (0.52)	0.11
Males (%)	28	76%	166	69%	214	69%	
BMI (kg/m^2)	37	29.9 (0.77)	241	30.4 (0.36)	309	30.0 (0.28)	0.67
Fasting glucose (mmol/L)	37	7.6 (0.38)	236	7.2 (0.12)	306	7.3 (0.10)	0.49
HbA_{1c} (%)	36	6.9 (0.22)	234	6.8 (0.08)	301	7.0 (0.08)	0.21
Cholesterol (mmol/L)	37	5.06 (0.16)	235	4.92 (0.06)	302	5.0 (0.06)	0.55
HDL-Cholesterol (mmol/L)	36	1.25 (0.09)	229	1.14 (0.02)	297	1.21 (0.02)	**0.03**
Triglycerides (mmol/L)	36	1.77 (0.20)	235	1.69 (0.06)	303	1.70 (0.06)	0.92
Creatinine (μmol/L)	35	80.1 (2.54)	228	79.1 (1.11)	290	79.6 (0.90)	0.90
Free Fatty Acids (μmol/L)	16	697 (39.3)	122	753 (21.1)	166	770 (17.1)	0.43
Insulin Sensitivity (M value) - $\mu mol \cdot min^{-1} \cdot m^{-2}$ BSA	35	686 (69.1)	238	646 (25.3)	305	666 (21.1)	0.76

N; numbers, M value; rate of whole body glucose metabolism during the euglycemic hyperinsulinemic (insulin dose: 40 $mU \cdot min^{-1} \cdot m^{-2}$ BSA) clamp. Bolded p values are < 0.05.

Supplementary Table 8: Oligonucleotides sequences.

CRISPR-Cas9 mutagenesis (*SLC30A8*-p.Arg138*)	5'-AGCAGGTACGGTTCATAGAG-3'
Primer pair 1	
	Forward: 5'-TACCCCAGGAATGGCTTCTC-3'
	Reverse: 5'-ACGTGTTCCTGTTGTCCCA-3'
Prime pair 2	
	Forward primer: 5'-AGTCTCTTCTCCCTGTGGTT-3'
	Reverse primer: 5'-ATGATCATCACAGTCGCCTG-3'
FAM probe (R138; CT)	5'-FAM-ATGGCACCGAGCTGA-MGB-3'
VIC probe (X138; TT)	5'-VIC-ATGGCACTGAGCTGAGA-MGB-3'
Prime pair 3	
	Forward primer: 5'-AGTCTCTTCTCCCTGTGGTT-3'
	Reverse primer: 5'-ATGATCATCACAGTCGCCTG-3'
CRISPR-Cas9 mutagenesis (*SLC30A8- p.Lys34Serfs50**)	5'-GTGAATAAAGATCAGTGTCC-3'
Primer pair 4	
	Forward: 5'-TGGTGGCATTGACTGAATAAGA-3'
	Reverse: 5'-ACCCTCCCATAATGATGCAGA-3'
	HDR-specific: 5'-GAAACCGGTGAATAGTGTCCCA-3'

References (For Supplemenatry Tables).

1. Abecasis, G.R., Cardon, L.R. & Cookson, W.O. A general test of association for quantitative traits in nuclear families. *Am. J. Hum. Genet.* **66**, 279-92 (2000).
2. Flannick, J. et al. Loss-of-function mutations in SLC30A8 protect against type 2 diabetes. *Nat Genet* **46**, 357-63 (2014).
3. Yang, J., Lee, S. H., Goddard, M.E. & Visscher, P. M. GCTA: a tool for genome-wide complex trait analysis. *Am J Hum Genet* **88**, 76-82 (2011).
4. Willer, C.J., Li, Y. & Abecasis, G. R. METAL: fast and efficient meta-analysis of genomewide association scans. *Bioinformatics* **26**, 2190-1 (2010).
5. Purcell, S. et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.* **81,** 559-75 (2007).

Supplementary Note

Methods:

Human study population.

Intravenous Glucose Tolerance Test (IVGTT, Extended Data Fig. 3): IVGTT group consists of a total of 849 (male-403, female- 446) individuals with an average age of 51 years. An antecubital polyethylene catheter was placed to one hand for the infusion of 0.3 g/kg body weight of glucose (maximum dose 35 g) intravenously for 2 minutes A retrogradely positioned wrist vein catheter was placed in the other hand, held in a heated (70°C) box in order to arterialize the venous blood. Arterialized blood samples were drawn at 0, 2, 4, 6, 8,10, 20, 30, 40, 50 and 60 minutes for P-glucose and S-insulin.

Biochemical measurements (Fig. 2, 3 and Extended Data Fig. 3a-b): P-glucose was analyzed using glucose oxidase (Beckman Glucose Analyzer, Beckman Instruments, Fullerton, CA, USA; Botnia Family Study) or glucose dehydrogenase method (Hemocue, Angelholm, Sweden; PPP-Botnia and test meal studies). In the Botnia Family study, S-insulin was measured by radioimmunoassay (RIA, Linco; Pharmacia, Uppsala, Sweden), enzyme immunoassay (EIA; DAKO, Cambridgeshire, U.K.) or fluoroimmunometric assay (FIA, AutoDelfia; Perkin Elmer Finland, Turku, Finland). For the analysis, insulin concentrations obtained with different assays were transformed to cohere with those obtained using the EIA. The correlation coefficient between RIA and EIA as well as between FIA and EIA was 0.98 ($P < 0.0001$). S-insulin was measured by the FIA in baseline visit of PPP-Botnia and the test meal study (correlation co-efficient 0.98). S-proinsulin was measured using RIA (Linco; Pharmacia, Uppsala, Sweden, OGTT data) or EIA (Mercodia AB, Uppsala, Sweden; test-meal data), and P-glucagon using RIA (EMD Millipore, St. Charles, MO; OGTT data) or EIA (Mercodia AB, Uppsala, Sweden; test-meal data). S-FFA was measured by an enzymatic colorimetric method (Wako Chemicals, Neuss, Germany). Serum total cholesterol, HDL and triglyceride concentrations were measured with Cobas Mira analyzer (Hoffman LaRoche, Basel, Switzerland), and since 2006 with an enzymatic method (Konelab 60i analyser; Thermo Electron Oy, Vantaa, Finland). Serum LDL cholesterol was calculated using the Friedewald formula. Blood collected in tubes containing DPP4-inhibitors was used for radioimmunoassay[1] for total P-GLP-1 (intact GLP-1 and the metabolite GLP-1 9-36 amide) during test meal.

Serum and urine samples for zinc were collected in trace element tubes (Beckton Dickinson, NJ, USA) and S- and U-zinc analyzed by two commercial laboratories: NordLab (Oulu, Finland; atom absorption spectrophotometry, AAS)

until 6th May 2015, then in Synlab (Helsinki, Finland; AAS for serum, mass spectrophotometry ICP-MS for U-zinc). The S-zinc concentrations were corrected for P-albumin (r=0.34, p=0.008 for Nordlab, r=0.34, p=0.03 for Synlab).

Genotyping: We analyzed genotype data for rs13266634 (p.Trp325Arg) and rs200185429 (p.Arg138*) for three cohorts genotyped with different genome-/exome-wide chips: the Botnia family cohort (Illumina Global Screening array-24v1, genotyped at Regeneron Pharmaceuticals), PPP-Botnia (Illumina HumanExome v1.1 array, genotyped at Broad Institute) and DIREVA (Illumina Human CoreExome array-24v1, genotyped at LUDC). For the Botnia family cohort, genotype data for p.Arg138* were imputed (info score >0.95) from the available GWAS data by phasing using SHAPT-IT v2[2] (http://mathgen.stats.ox.ac.uk/genetics_software/shapeit/shapeit.html) and imputing using the GoT2D reference panel[3] by IMPUTEv2[4] (http://mathgen.stats.ox.ac.uk/impute/impute_v2.html). The carrier status of all 20 imputed p.Arg138* from Botnia family cohort was additionally confirmed from available exome sequencing data. Genotyping (p.Trp325Arg and p.Arg138*) the family members participating in the genotype based recall study (test meal study) was performed using TaqMan (Applied Biosystems, Carlsbad, CA) and additionally genotyped by Illumina Global Screening array-24v1. The genotype distribution of both variants was in accordance with Hardy-Weinberg equilibrium in all the cohorts. We did not detect any Mendelian errors in the families.

Study participants and their clinical measurements in the Verona Newly Diagnosed Diabetes Study (VNDS, Extended Data Fig. 3c): The Verona Newly Diagnosed Type 2 Diabetes Study (VNDS; NCT01526720) is an ongoing study aiming at building a biobank of patients with newly diagnosed (within the last six months) type 2 diabetes. Patients are drug-naïve or, if already treated with antidiabetic drugs, undergo a treatment washout of at least one week before metabolic tests are performed[5]. Each subject gave informed written consent before participating in the research, which was approved by the Human Investigation Committee of the Verona City Hospital. Metabolic tests were carried out on two separate days in random order[5] .

Plasma glucose concentration was measured in duplicate with a Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA, USA) or an YSI 2300 Stat Plus Glucose &Lactate Analyzer (YSI Inc., Yellow Springs, OH, USA) at bedside. Serum C-peptide and insulin concentrations were measured by chemiluminescence as previously described[5]**.** The analysis of the glucose and C-peptide curves during the OGTT was carried out with a mathematical model as described previously[5]. This model was implemented in the SAAM 1.2 software (SAAM Institute, Seattle, WA) to estimate its unknown parameters. Numerical values of the unknown parameters were estimated by using nonlinear least squares. Weights were chosen optimally, i.e., equal to the inverse of the variance of the measurement errors, which were assumed to be additive, uncorrelated, with zero mean, and a coefficient of variation (CV) of 6-8%. A good fit of the model to data was obtained in all cases and unknown parameters were estimated with good precision. In this paper

we report the response of the beta cell to glucose concentration (proportional control of beta cell function), which in these patients accounts for 93.2±0.3% of the insulin secreted by the beta cell in response to the oral glucose load. Genotypes were assessed by the high-throughput genotyping Veracode technique (Illumina Inc, CA), applying the GoldenGate Genotyping Assay according to manufacturer's instructions. Hardy-Weinberg equilibrium was tested by chi-square test. Variant association analyses were carried out by generalized linear models (GLM) as implemented in SPSS 25.0 and they were adjusted for a number of potential confounders, including age, sex and BMI.

iPSC generation, differentiation and genome editing (Fig. 4, Extended Data Fig. 4 and 5).

CRISPR-Cas9 mediated generation of p.Lys34Serfs50* human induced pluripotent stem cell line: To generate *SLC30A8*-p.Lys34Serfs50* the gRNA addressing the target site for CRISPR/Cas9 mutagenesis (Supplementary Table 8) was synthesized using the Engen sgRNA synthesis kit (NEB) according to manufacturer's instruction. 20 uM of synthesized gRNA was reconstituted with 20 uM of Cas9 protein (NEB) and incubated at room temperature for 15 minutes to form a ribonucleoprotein (RNP) preparation of CRISPR/Cas9. A single strand oligonucleotide repair template for HDR containing the required seven nucleotide deletions (c.101_107del, p.Lys34Serfs50*) was synthesized (Eurogentec). Human iPSCs ($1x10^5$ cells) were electroporated with the RNP preparation and 50 uM of the repair template with the Neon Transfection System from ThermoFisher Scientific using 10 uL tips (1200 volts, 30 ms, 2 pulses) according to manufacturer's guidelines. Following electroporation and single cell plating, single clones were picked and expanded as described previously. Genotyping PCR was performed using primers (primer pair 4, Supplementary Table 8) to amplify the target regions within exon 2 (511/504 bp) and the HDR-repaired allele (244 bp). Successfully targeted clones were confirmed via Sanger sequencing and monoallelic sequencing and passed quality control checks. From 96 clones, 11 clones were heterozygous for p.Lys34Serfs50* but also contained indels in the other allele. Another 11 clones were homozygous for the variant, of which two were selected (clone B3 and D3).

In vitro differentiation of hiPSCs towards Beta-like cells:

<u>*Stage 1 (Definitive Endoderm):*</u> Cells were washed once with PBS before adding 0.5% bovine serum albumin (BSA; Roche #10775835001) MCDB131 media [(ThermoFisher Scientific #10372019) containing 1x Penicillin-Streptomycin (Sigma #P0781), 1.5 g/L sodium bicarbonate (ThermoFisher Scientific #25080060), 1x GlutaMAX™ (ThermoFisher Scientific #35050038) and 10 mM Glucose (ThermoFisher Scientific #A2494001)] supplemented with 100 ng/mL Activin A (Peprotech #120-14) and 3 μM CHIR 99021 (Axon Medchem #1386). On day 2 and 3, cells were cultured with 0.5% BSA MCDB131 media supplemented with either 100 ng/mL Activin A and 0.3 μM CHIR 99021 (day 2) or with 100 ng/mL Activin A alone (day 3).

Stage 2 (Primitive Gut Tube): Cells were cultured for 48 hours in 0.5% BSA MCDB131 media with 0.25 mM ascorbic acid (Sigma #A4544) and 50 ng/mL KGF (PeproTech #100-19).

Stage 3 (Posterior Foregut): Cells were cultured for two days in 2% BSA MCDB131 media supplemented with 1 g/L sodium bicarbonate, 0.25 mM ascorbic acid, 0.5x Insulin-Transferrin-Selenium-Ethanolamine (ITS-X; ThermoFisher Scientific #51500056), 1 μM retinoic acid (RA; Sigma-Aldrich #R2625), 0.25 μM Sant-1 (Sigma-Aldrich #S4572), 50 ng/ml KGF, 100 nM LDN193189 (Stemgent #04-0074), and 100 nM α-Amyloid Precursor Protein Modulator (Merck #565740).

Stage 4 (Pancreatic Endoderm): Cells were cultured for three days in 2% BSA MCDB131 media supplemented with 1 g/L sodium bicarbonate, 0.25 mM ascorbic acid, 0.5x ITS-X, 0.1 μM RA, 0.25 μM Sant-1, 2 ng/ml KGF, 200 nM LDN193189 and 100 nM α-Amyloid Precursor Protein Modulator.

Stage 5 (Endocrine Progenitors): Cells remained in planar culture for three days in 2% BSA MCDB131 media supplemented with 20 mM final glucose, 0.5x ITS-X, 0.05 μM RA, 0.25 μM Sant-1, 100 nM LDN193189, 10 μM ALK5 Inhibitor II (Enzo Life Sciences #ALX-270-445), 1 μM 3,3,5-Triiodo-L-thyronine sodium salt (T3; Sigma-Aldrich #T6397), 10 μM zinc sulfate heptahydrate (Sigma # Z0251), and 10 μg/mL heparin sodium salt (Sigma #H3149).

Stage 6 (Endocrine Cells): Cells remained in planar culture for six days in 2% BSA MCDB131 media supplemented with 20 mM final glucose, 0.5x ITS-X, 100 nM LDN193189, 10 μM ALK5 Inhibitor II, 1 μM T3, 10 μM zinc sulfate heptahydrate, and 100 nM γ-Secretase Inhibitor XX (Merck Millipore #565789).

Stage 7 (Beta-like Cells): Cells remained in planar culture for another six days in 2% BSA MCDB131 media supplemented with 20 mM final glucose, 0.5x ITS-X, 10 μM ALK5 Inhibitor II, 1 μM T3, 1 mM N-Cys (Sigma-Aldrich #A9165), 10 μM Trolox (EMD Millipore #648471), 2 μM R248 (SelleckChem #S2841), and 10 μM zinc sulfate heptahydrate.

Allele-specific SLC30A8 expression by targeted RNA sequencing of SLC30A8 (Figure 4e and Extended Data Fig. 5c): Dual-indexed RNA libraries were prepared with target specific priming of both strand synthesis from 50 ng of extracted RNA from p.Arg138* edited (clone A3 and B1) and unedited Beta-like Cells derived from hiPSC using QuantSeq-Flex Targeted RNA-Seq Library Prep Kit V2 (Lexogen GmbH, Vienna, Austria) according to user guide version 015UG058V0230. Targeted primers covering p.Arg138* mutation (primer pair 3, Supplementary Table 8) were generated and further modified with partial Illumina P7 and P5 adapter extensions. For normalization of the data, 6 base pair unique molecular identifier (UMI) was included into first strand synthesis primer. Quality of libraries was

measured using 2100 Bioanalyzer DNA High Sensitivity Kit (Agilent, Santa Clara, CA, USA). Linker-linker artifacts were removed with BluePippin DNA size-selection (Sage Science Inc., Beverly, MA, USA) before pooling the libraries to the sequencing run. Sequencing (2x250 bp) was performed with Illumina MiSeq system using v2 chemistry (Illumina, San Diego, CA, USA). Target RNA sequencing reads were aligned to hg38 using STAR (Spliced Transcripts Alignment to Reference)[6,7] and UMI-tools[8] used to remove PCR based duplications (deduplication) using 6 bp UMI in read 2.

SLC30A8 and INS transcript expression in hiPSC-derived BLCs by RNAscope® (Extended Data Fig. 4e-g): Stage 7 cells were trypsinized and the resulting single cell suspension was subjected to cytospin (~50,000 cells/slot) for 5 minutes at 1200 rpm. Samples were then fixed in 10% neutral buffered formalin for 40 minutes at 37°C, washed once in PBS and slides were dehydrated for 5 minutes at room temperature in 50%, 70%, and 2x5 minutes in 100% ethanol before storage at -20°C. Before proceeding with the RNAScope® In Situ Hybridization Technology (Advanced Cell Diagnostics, Inc.), samples were rehydrated and processed following the manufacturer's recommendations for cultured adherent cell sample preparation using the RNAscope® Multiplex Fluorescent Reagent Kit_v2 (ACDbio#323100). For hybridization, RNAscope® Probe-Hs-*SLC30A8* (ACDbio#441261) and RNAscope® Probe-Hs-*INS*-C2 (ACDbio#313571-C2) were used. For detection, Cy3 fluorophore (PerkinElmer TSA Plus Cyanine 3 System #NEL744E001KT) was used to detect *SLC30A8* and Cy5 (PerkinElmer TSA Plus Cyanine 5 System #NEL745E001KT) was used to detect *INS* at 1:1500 dilution. Samples were counterstained with DAPI to detect nuclei and mounted with ProLong Gold Antifade Mountant (Thermofisher). A minimum of six independent fields were captured using a Nikon Eclipse TE2000-U Epifluorescence microscope with a plan fluor ELWD 20x ADL objective. Independent images were analyzed using QuPath v0.1.2 and the cell mean intensity for each was measured. Once background was subtracted, cells with values of *SLC30A8*>10 were included.

Western blot of ZnT8: Cell pellets were lysed in RIPA buffer (50mM Tris pH 7.4, 150mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS) containing 1x protease inhibitor cocktail (Roche). Protein samples were prepared in Laemmli buffer and not heat denatured. 10µg of protein were loaded on a Mini-PROTEAN TGX 4-20% precast gel (Bio-Rad) and run at 300V for 15minutes. The gel was activated on a ChemiDoc MP Imaging System and transferred to a Trans-Blot Turbo polyvinylidene fluoride (PVDF) membrane using the Trans-Blot Turbo Transfer System (all Bio-Rad). Membranes were blocked in 5% milk for 1h at RT, incubated with primary antibody against Znt8 ((1), 1:1000) overnight at 4°C followed by 1h incubation at RT with HRP-coupled secondary anti-mouse IgG antibody (Thermo Scientific 31450, 1:2500). The membranes were subsequently incubated for 4 min. at RT with Clarity Western enhanced chemiluminescence (ECL) reagent and imaged on the ChemiDoc MP Imaging System (Bio-Rad). Western

Blot images were quantified using Image Lab software (Bio-Rad) and normalised to a loading control on the same blot (β-tubulin (Santa Cruz, sc-365791, 1:2000).

EndoC-βH1 culture (Fig. 5).

Insulin and proinsulin secretion and content (Helsinki): For the measurement of secreted insulin or proinsulin in the supernatant, 96h post-transfected cells were washed twice with 1X PBS and incubated with fresh EndoC-βH1 culture medium for next 24h. Secreted and intracellular insulin and proinsulin were measured using a commercial human insulin Elisa and human proinsulin Elisa kit from Mercodia (Mercodia AB, Uppsala, Sweden). Proinsulin to insulin ratio was calculated by dividing the respective values measured from the supernatant and the cells (pmol/L).

Immunoblotting (Helsinki and Oxford): Total cellular protein was prepared with Laemmli buffer and resolved using Any kD Mini-Protean-TGX gel (Bio-Rad). Immunoblot analysis was performed by overnight incubation of with primary antibodies against PC1/3 (Cell Signaling; #11914; 1:1000), CPE (BD Bioscience; #610758; 1:1000), PC2 (Santa-Cruz; #SC-374140; 1:450), Phospho-AKT-Ser473 (Cell Signaling; #4060; 1:1000) and AKT (Santa-Cruz; #SC-8312; 1:500). The membranes were further incubated with species-specific HRP-linked secondary antibodies (1:5000) and visualization was performed following ECL exposure with ChemiDoc XRS+ system and Image Lab Software (Bio-Rad). A loading control of either alpha-Tubulin (Sigma; T5168; 1:5000) or beta-actin (Sigma; A5441; 1:5000) was performed on the same blot for all western blot data. Densitometric analysis of bands from image were calculated using Image J (Media Cybernetics) software and intensities compared as PC1/3, phosphor-AKT-Ser473, PC2 to tubulin; CPE to beta-actin. Western blot for ZnT8 was performed as described previously in iPSC section.

Cell viability assay(MTT): EndoC-βH1 cells were transfected with either siScramble or siSLC30A8 for 96h. The viability of cells after 24 h of tunicamycin (10 μg/ml) treatment was determined using Vybrant MTT Cell proliferation kit (ThermoFisher Scientific; #M6494), the standard MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. All the treatments were performed on cells with equal seeding density (5×10^4 cells/well) in 96 wells plate. The purple formazan crystals generated after 2 h incubation with MTT buffer were dissolved in DMSO, and the absorbance was recorded on a microplate reader at a wavelength of 540nm.

***Zinpyr-1 based Zinc staining in EndoC-βH1 cells (Helsinki)*:** EndoC-βH1 cells, 1.5 x 10^5 cells per well of 24-well plate (Costar #3526) were treated with siRNA against *SLC30A8* or Non-targeted control as described previously in siRNA methods section. After 96 h siRNA treatment, cells were washed twice with KRBH complete buffer containing 5.5mM glucose and load with zinc-specific fluorescent dye Zinpyr-1 (5μM, Cayman Chemicals #15122) for 30 minutes in the cell culture incubator[9]. Then, fluorescent images were obtained after rinsed in KRBH buffer, with an IncuCyte-S3

Live-Cell Imaging system (Essen BioScience) using 488 nm laser. Images were analyzed with IncuCyte S3 software and presented as ratio of green mean intensity object average to phase area confluency.

Assessment of granule zinc content: monitoring of stimulated zinc secretion using ZIMIR (Extended Data Fig. 6): EndoC-βH1 cells were seeded on glass slides and transfected with siRNA control or targeted against *SLC30A8* (SMARTpool ON-targetplus) at a final concentration of 20nM using Lipofectamine RNAiMax (Thermo Fisher) according to manufacturer's instruction. 72 h post-transfection, cells were incubated for 2 h in culture media containing 3mM glucose and then for 20 minutes in KHB buffer saturated with 95% O_2/5% CO_2 and adjusted to pH 7.4, containing 3 mM glucose and 5μM Zinc binding probe zinc indicator for monitoring induced exocytotic release (ZIMIR[10]). Cells were then transferred to an imaging chamber and acquisitions were performed in KHB buffer with KCl added (20mM final concentration) 3 minutes after acquisition began.

Total internal reflection of fluorescence (TIRF) imaging was performed on a Nikon Eclipse Ti microscope equipped with a 100x/1.49NA TIRF objective, a TIRF/FRAP iLas2 module to control laser angle (Roper Scientific), a Quad Band TIRF Filter Cube (TRF89902 – Chroma) and an ibidi heating system. ZIMIR was excited using a 488 nm laser line, and images were acquired with an ORCA-Flash 4.0 camera (Hamamatsu). Metamorph software (Molecular Devices) was used for data capture. Image analysis was performed using ImageJ, to measure fluorescence intensity close to (within ~70 nm of) the plasma membrane. Traces are presented as normalized intensity over time (F/F0).

RNA (mRNAs) sequencing of EndoC-βH1 cells (Extended Data Fig. 7): For RNA sequencing post 96h siScramble (n=8) or siSLC30A8 (n=8) transfected EndoC-βH1 cells were used and the total RNA was extracted with Macherey-Nagel RNA isolation kit as per manufacturer's instruction. RNA sequencing was performed using Illumina TruSeq-mRNA library on NextSeq 500 system (Illumina) with an average of >15 million paired-end reads (2 × 75 base pairs). RNA sequencing reads were aligned to hg38 using STAR (Spliced Transcripts Alignment to Reference)[6], genome annotations were obtained from the GENCODE (Encyclopedia of Genes and Gene Variants) v22[7] program, and reads counting were done using featureCounts[11]. Further downstream analysis was perform using edgR[12] software package, low expressed (<-1 median log 2 transformed counts per gene) genes were removed, read counts were normalized using TMM[13] (trimmed mean of M-values), differential expression analysis was performed using method similar to Fisher's Exact Test (as implemented in edgR) and corrected for multiple testing using Bonferroni method. Over-representation analysis among differentially expressed genes against 12 different pathway data bases (Reactome, BioCarta, KEGG, Wikipathways, EHMN, HumanCyc, INOH, NetPath, PharmGKB, PID, Signalink, SMPDB), as implemented in ConsensusPathDB (Release 34, http://cpdb.molgen.mpg.de/)[14], was performed using a hypergeometric test and corrected for multiple correction (FDR correction).

The gene set enrichment analysis was performed using GSEA software (GSEA vs 3.0)[15] against gene ontology database (c5.all.v6.2.symbols.gmt) with 1000 permutations.

Expression of p.Arg138* mutation in INS1E (Extended Data Fig. 8).

INS-1E cells[16] were used for transient transfection of pcDNA3.1(+)-p.Arg138* construct fused to fluorescent m-Cherry at C-terminus using transfection reagent Viromer according to the manufacturer's instructions. After transfections cells were collected at 24, 48, 72 and 96 hours and analyzed by western blot analysis using mCherry (600-401-P16, Rockland) antibody. Untransfected cells were used as control and tubulin as a loading control. Two days after transient transfections with either p.Arg138*-mCherry (INS1E), p.Arg138*-HA or p.Arg138*-Myc-His construct (INS1E), cells were washed with PBS twice and fixed using 4% paraformaldehyde for 15 minutes at room temperature. Cells were permeabilized with 0.2 % Triton X-100 in phosphate-buffered saline (PBS) for 10 mins and to prevent unspecific binding were further blocked for 1 h with 5% FBS in PBS. INS1E cells transfected with either p.Arg138*-HA or p.Arg138*-Myc-His construct were incubated with the primary antibody (HA antibody: MMS-101P, Biolegend; His antibody: D291-A48, MBL; insulin antibody: A0564, DAKO), overnight at 4°C. Secondary antibodies were conjugated to Alexa Fluor 488 (Molecular Probes). Cells transfected with mCherry construct were imaged after 48 and 96 hours (INS1E) in order to visualize subcellular localization at different time points.

Measurements of cytosolic zinc in INS-1(832/13) cells (Extended Data Fig. 8).

Cell culture: INS-1 (823/13) cells were grown in RPMI 1640 medium (Sigma-Aldrich, UK) supplemented with 10% (v/v) foetal bovine serum (FBS), 2 Mm L-glutamine, 0.05 mM 2-mercaptoethanol, 10 mM HEPES (Sigma-Aldrich), 1 mM sodium pyruvate (GIBCO, France), 2 mM L-glutamine and antibiotics (100 µg/ml Streptomycin and 100 U/ml penicillin). Cells were maintained in 95% oxygen, 5% carbon dioxide at 37°C.

Co-transfection: Cells were seeded on sterile coverslips at 60% confluence and co-transfected using lipofectamine 2000 (Invitrogen, USA) according to the manufacturer's instructions, with either the empty construct (EV) or the rare-truncated variant (c-Myc tag, R138X) construct and the Förster Resonance Energy transfer sensors (FRET), eCALWY-4 vector (free cytosolic zinc measurements).

Protein extraction and Western (immuno-) blotting analysis: For protein extraction, RIPA buffer (1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 0.15 mM NaCl, 0.01 M sodium Phosphate pH7.2) was used for lysis. Protein extracts were resolved in SDS-page (12% vol/vol acrylamide) and transferred to a polyvinylidene fluoride (PVDF) membrane, followed by blocking for 1 hour, immunoblotting with either c-Myc anti-mouse SLC30A8 (1:400) and the secondary anti-mouse antibody (1:10000, Abcam), and then the mouse monoclonal anti-tubulin (1:10000) and

secondary anti-mouse for tubulin (1:5000). Chemiluminescence detection reagent (GE Healthcare) was used before exposing to hyperfilms.

Immunocytochemistry: Cells were fixed in 4% (v/v) Phosphate-buffered saline/Paraformaldehyde (PFA). Cells were permeabilized in 0.5% (w/v) PBS/TritonX-100 and further saturated with PBS/BSA 0.1%. Cells were then incubated for 1 hour with the primary antibody, anti-c-Myc mouse antibody (1:200) followed by the secondary Alexa Fluor® 568 nm anti-mouse IgG (H+L, 1:1000 Life Technologies, USA). Coverslips were mounted with mounting medium containing DAPI (Vectashield, USA) on microscope slides (ThermoScientific). Imaging was performed on a Nikon Eclipse Ti microscope equipped with a 63x/1.4NA objective, spinning disk (CAIRN, UK) using a 405, 488 and 561 nm laser lines, and images were acquired with an ORCA-Flash 4.0 camera (Hamamatsu) Metamorph software (Molecular Device) was used for data capture.

Cytosolic free Zn^{2+} measurements: Cells were co-transfected with R138X (p.Arg138*) construct or empty construct (EV) and eCALWY-4 construct. Acquisitions were performed 24 hours after transfection using an Olympus IX-70 wide-field microscope with a 40x/1.35NA oil immersion objective and a zyla sCMOS camera (Andor Technology, Belfast, UK) controlled by Micromanager software. Excitation was provided at 433 nm using a monochromator (Polychrome IV, Till Photonics, Munich, Germany). Emitted light was split and filtered with a Dual-View beam splitter (Photometrics, Tucson, Az, USA) equipped with a 505dcxn dichroic mirror and two emission filters (Chroma Technology, Bellows Falls, VT, USA - D470/24 for cerulean and D535/30 for citrine). Cells were perfused for 4 minutes with KREBS buffer (140 mM NaCl, 3.6 mM KCl, 0.5 mM NaH_2PO_4, 0.2 mM $MgSO_4$, 1.5 mM $CaCl_2$, 10 mM HEPES, 25 mM $NaHCO_3$) without additives, next the buffer was changed to KREBS buffer containing 50 μM N,N,N',N'-tetrakis(2-pyridylmethyl)ethylenediamine (TPEN, Sigma) for 5 minutes, followed by perifusion with KREBS buffer containing 100 μM $ZnCl_2$ and 5 μM of the Zn^{2+}-specific ionophore 2-mercaptopyridine N-oxide (Pyrithione, Sigma). Image analysis was performed using ImageJ software. Steady-state fluorescence intensity ratio of acceptor over donor was measured, followed by the determination of the minimum and maximum ratios to calculate the free Zn^{2+} concentration using the following formula: $[Zn^{2+}] = Kd \cdot ((R - Rmin)/(Rmax - R))$, in which Rmin is the ratio in the Zn^{2+} depleted state, after addition of 50 μM TPEN, and Rmax was obtained upon Zn^{2+} saturation with 100 μM $ZnCl_2$ in the presence of 5 μM pyrithione.

Human Pancreatic Islets (Fig. 7).

Human pancreatic islets from LUDC: The Association of p.Trp325Arg genotype with expression of *SLC30A8* and other candidate genes was performed using RNA sequencing from islets of 139 non-diabetic individuals as described

previously[17,18]. Briefly, RNA sequencing of islets was done using a HiSeq 2000 system (Illumina) for an average depth of 32.4 million paired-end reads (2 × 100 base pairs)[17,18]. RNA sequencing reads were aligned to hg19 using STAR (Spliced Transcripts Alignment to Reference)[6]. Genome annotations were obtained from the GENCODE (Encyclopedia of Genes and Gene Variants) v20[7] program and read counting was done using featureCounts[11]. Read counts were normalized to total reads (counts per million) and additionally across-samples normalization was done using TMM method[13]. Association analysis (so called eQTL) was performed on inverse normalized expression values using linear regression adjusted for age, sex and islets purity using PLINK[19].

References (For Supplementary Note).

1. Lindgren, O. et al. Incretin hormone and insulin responses to oral versus intravenous lipid administration in humans. *J. Clin. Endocrinol. Metab.* **96**, 2519-24 (2011).
2. Delaneau, O., Zagury, J. F. & Marchini, J. Improved whole-chromosome phasing for disease and population genetic studies. *Nat. Methods* **10 (1),** 5-6 (2013).
3. Flannick, J. et al. Sequence data and association statistics from 12,940 type 2 diabetes cases and controls. *Sci. Data.* **4**, 170179 (2017).
4. Howie, B., Fuchsberger, C., Stephens, M., Marchini, J. & Abecasis G. R. Fast and accurate genotype imputation in genome-wide association studies through pre-phasing. *Nat. Genet.* **44**, 955-9 (2012)
5. Bonetti, S. et al. Variants of GCKR affect both β-cell and kidney function in patients with newly diagnosed type 2 diabetes: the Verona newly diagnosed type 2 diabetes study 2. *Diabetes Care* **34**, 1205-10 (2011).
6. Dobin, A. et al. STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* **29**, 15-21 (2013).
7. Harrow, J. et al. GENCODE: the reference human genome annotation for The ENCODE Project. *Genome Res.* **22**, 1760-74 (2012).
8. Smith, T., Heger, A. & Sudbery, I. UMI-tools: modeling sequencing errors in Unique Molecular Identifiers to improve quantification accuracy. *Genome Res.* **27,** 491-499 (2017).
9. Slepchenko, K. G. et al. Autocrine effect of Zn^{2+} on the glucose-stimulated insulin secretion. *Endocrine* **50**, 110-22 (2015).
10. Li, D. et al. Imaging dynamic insulin release using a fluorescent zinc indicator for monitoring induced exocytotic release (ZIMIR). *Proc. Natl. Acad. Sci. USA* **108**, 21063-8 (2011
11. Liao, Y., Smyth, G. K. & Shi, W. featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics* **30**, 923-30 (2014).
12. Robinson, M. D., McCarthy, D. J. & Smyth G. K. "edgeR: a Bioconductor package for differential expression analysis of digital gene expression data." *Bioinformatics* **26**, 139-140 (2010).
13. Robinson, M. D. & Oshlack, A. A scaling normalization method for differential expression analysis of RNA-seq data. *Genome Biol.* **11**, R25 (2010).
14. Herwig, R. et al. Analyzing and interpreting genome data at the network level with ConsensusPathDB. *Nat. Protoc.* **11**, 1889-907 (2016).
15. Mootha, V. K. et al. PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. *Nat. Genet.* **34**, 267-73 (2003).
16. Asfari, M. et al. Establishment of 2-mercaptoethanol-dependent differentiated insulin-secreting cell lines. *Endocrinology* **130,** 167-78 (1992).
17. Ottosson-Laakso, E. et al. Glucose-Induced Changes in Gene Expression in Human Pancreatic Islets: Causes or Consequences of Chronic Hyperglycemia. *Diabetes* **66**, 3013-3028 (2017).
18. Fadista, J. et al. Global genomic and transcriptomic analysis of human pancreatic islets reveals novel genes influencing glucose metabolism. *Proc. Natl. Acad. Sci. U S A* **111**, 13924-9 (2014).
19. Purcell, S. et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.* **81,** 559-75 (2007).