Loss of ZnT8 function protects against diabetes by enhanced insulin secretion

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Abstract

A rare loss-of-function allele p.Arg138* in SLC30A8 encoding the zinc transporter 8 (ZnT8), enriched in Western Finland, protects against type 2 diabetes (T2D). We recruited relatives of the identified carriers and showed that protection was associated with better insulin secretion due to enhanced glucose responsiveness and proinsulin conversion, particularly when compared with individuals matched for the genotype of a common T2D-risk allele in SLC30A8, p.Arg325. In genome-edited human iPSC-derived β-like cells, we establish that the p.Arg138* allele results in reduced SLC30A8 expression due to haploinsufficiency. In human β-cells, loss of SLC30A8 leads to increased glucose responsiveness and reduced K<sub>ATP</sub> channel function similar to isolated islets from carriers of the T2D-protective allele p.Trp325. These data position ZnT8 as an appealing target for treatment aimed at maintaining insulin secretion capacity in T2D.
Zinc transporters (ZNT) regulate the passage of zinc across biological membranes out of the cytosol, while Zrt/Irt-like proteins (ZIP) transport zinc into the cytosol. ZnT8, encoded by SLC30A8, is highly expressed in the membrane of insulin granules within pancreatic β-cells, where it transports zinc ions for crystallization and storage of insulin. We previously described a loss-of-Function (LoF) allele p.Arg138* (rs200185429, c.412C>T) in the SLC30A8 gene that conferred 53% protection against T2D. This allele was extremely rare (0.02%) in most European countries but more common (>0.2%) in Western Finland. We also reported a protective frameshift allele p.Lys34Serfs50* conferring 83% protection against T2D in Iceland. Further, the SLC30A8 gene harbors a common variant (rs13266634, c.973T>A) p.Trp325Arg in the C-terminal domain. Whilst the major p.Arg325 allele (>70% of the population) confers increased risk for T2D, the minor p.Trp325 allele is protective.

The mechanisms by which modulation of ZnT8 activity protects against T2D are largely unknown. Several attempts have been made to study loss of Slc30a8 function in rodent models, but the results have been inconclusive; global knock-out of Slc30a8 led to either glucose intolerance or had no effect in mice, whilst over-expression improved glucose tolerance without effect on insulin secretion. A mouse model harboring the equivalent of the human p.Arg138* allele lacked any detectable ZnT8 protein but showed no effect on glucose tolerance. These rodent studies present a complex picture that may not recapitulate the T2D protective effects of SLC30A8 LoF alleles in humans. We therefore performed detailed metabolic studies in human carriers heterozygous for the LoF allele (p.Arg138*) recruited on the basis of their genotype, performed comprehensive functional studies in human β-cell models, and compared these with the mouse model carrying the human p.Arg138*-Slc30a8 allele.
Results

Recruitment by genotype

Given the enrichment of the p.Arg138*-SLC30A8 allele in Western Finland, we genotyped >14,000 individuals from the Botnia Study\textsuperscript{11} for the SLC30A8 p.Arg138* and the common p.Trp325Arg variants, and identified 71 p.Arg138*-SLC30A8 carriers (all heterozygotes; 55 non-diabetic individuals, Fig. 1). We then recruited family members of known p.Arg138* carriers to identify additional p.Arg138* carriers to perform a detailed metabolic study (190 minutes test meal) in carriers and non-carrier relatives. Of the 79 p.Arg138* carriers (65 novel, 14 previously identified) and 103 non-carrier relatives from >21 families (Supplementary Fig. 1), 54 and 47, respectively, participated in a test meal and 31 and 13 participated in an oral glucose tolerance test (OGTT) during a separate second visit (Fig. 1, Supplementary Table 1 and 3). We also had data from previously performed OGTTs within the Botnia Study for 8436 non-diabetic individuals (55 p.Arg138* carriers, Fig. 1, Supplementary Table 2 and 3). Of the 136 p.Arg138* allele carriers, none were homozygous for the protective common variant, p.Trp325, and p.Arg138* segregated with p.Arg325 in all families (Supplementary Fig. 1). Thus, we present the data in three different ways: 1) p.Arg138* vs. all p.Arg138Arg, 2) p.Arg138* vs. p.Arg138Arg having at least one p.Arg325 allele (p.Trp325Arg or p.Arg325Arg), and 3) p.Arg325 (p.Trp325Arg or p.Arg325Arg) vs. p.Trp325Trp on a background of p.Arg138Arg.

Replicating our previous findings\textsuperscript{3}, carriers of p.Arg138* had a reduced risk of T2D (OR=0.40, p=0.003) when analyzing 4564 T2D (13 p.Arg138* carriers) and 8183 non-diabetic (55 p.Arg138* carriers) individuals. Additionally, non-diabetic p.Arg138* carriers had lower fasting glucose concentrations than p.Arg138Arg individuals (Supplementary Table 5 and 6). There were no significant differences in plasma zinc concentrations measured during test meal or OGTT between the groups (data not shown).
Comparison of p.Arg138* vs. p.Arg138Arg: The p.Arg138* carriers had lower blood glucose levels during the test meal especially during the first 40 minutes (area under curve, p=0.02) and showed a higher corrected insulin response (CIR) (at 20 minutes, p=0.046) than non-carriers (Supplementary Tables 4). Similarly, in the larger population-based OGTT cohort, carriers had higher insulin response during OGTT (Fig 3b-c, left panel), especially with respect to the early incremental insulin response (p=0.008) and the insulin/glucose ratio (at 30 minutes, p=0.002, Supplementary Table 5).

The higher insulin secretory response during OGTT was consistent across different subsets with OGTT data (meta-analysis: CIR, P=0.002; incremental insulin, p=2.4×10⁻⁴; 30 minutes insulin, p=3.8×10⁻⁴) (Supplementary Table 6). Of note, the p.Arg138* carriers had significantly lower proinsulin/C-peptide (20 minutes: p=0.041; 40 minutes: p=0.043) and proinsulin/insulin (20 minutes: p=0.006) ratios during the test meal suggesting effects on proinsulin conversion (Fig. 2d-e). No differences were seen in glucagon, GLP-1 or free fatty acid concentrations during the test meal (Supplementary Fig. 2c-e). Neither model-based insulin clearance index nor the ratio of insulin and C-peptide areas under the curve during the test meal differed between p.Arg138* and p.Arg138Arg (Supplementary Fig. 2f-g).

Comparison of p.Arg138* vs. p.Arg138Arg–p.Arg325: The above differences were magnified when we restricted the p.Arg138Arg group to carriers of the common risk variant p.Arg325 (middle panel of Fig. 2). The early phase (0-40 minutes) insulin (p=0.026), insulin/glucose ratio (p=0.004) and CIR (p=0.004; 20 minutes) were all greater in p.Arg138* carriers vs. p.Arg138Arg on a background of p.Arg325 (Supplementary Table 4). Both the proinsulin/C-peptide (20 minutes: P=0.027, 40 minutes: P=0.044) and proinsulin/insulin ratios (20 minutes: P=0.003) were reduced in p.Arg138* carriers (middle panel of Fig. 2d-e).

Comparison of p.Trp325Trp vs. p.Arg325: The effect of p.Trp325Trp genotype on glucose and insulin response mimicked the effects of p.Arg138* with pronounced early (20 minutes) insulin (p=0.032) and C-peptide (p=0.030) responses during the test meal (right panel of Fig. 2b-c and
Supplementary Fig. 2a), as well as increased insulin secretion (30 minutes insulin, 30 minutes insulin/glucose, incremental insulin, p≤0.003), lower fasting and 120 minute proinsulin (p=0.006 and p=0.039, respectively) concentration during OGTT in p.Trp325 carriers (Supplementary Table 5, right panel of Fig. 3b-c). Moreover, p.Trp325Trp carriers showed a pronounced increased (p=0.003) early incremental insulin secretion during the intravenous glucose tolerance test (IVGTT) (Supplementary Fig. 3a-b and Supplementary Table 5). In patients with newly diagnosed T2D (Supplementary Table 7), the p.Trp325Trp carriers showed a trend (p=0.12) towards enhanced β-cell sensitivity to glucose during the OGTT (Supplementary Fig. 3c).

Taken together, all human in vivo data on p.Arg138* show enhanced glucose-stimulated insulin secretion combined with enhanced proinsulin conversion as a potential explanation for T2D protection. The common allele p.Trp325 is also associated with protection from T2D and the metabolic effects are similar but more modest than those of on p.Arg138*, suggesting it also might reduce ZnT8 function.

**SLC30A8 p.Arg138* and p.Lys34Serfs50* variant in human iPSCs**

The majority of nonsense SLC30A8 alleles (including p.Arg138* and p.Lys34Serfs50*) which protect against T2D are located in the first four exons of the eight-exon canonical islet SLC30A8 transcript (ENST00000456015) and are predicted to undergo nonsense mediated decay (NMD), a cell surveillance pathway that reduces errors in gene expression by eliminating mRNA transcripts with premature stop codons. To confirm that nonsense SLC30A8 alleles lead to haploinsufficiency through NMD, we used CRISPR-Cas9 to introduce two protective alleles, p.Arg138* and p.Lys34Serfs50*, into the SLC30A8 locus of the SB Ad3.1 human iPSC cell line (Supplementary Fig. 4a). Two heterozygous hiPSC lines for the SLC30A8-p.Arg138* allele (clone B1 and A3) and two homozygous hiPSC lines for SLC30A8-p.Lys34Serfs50* (clone B3 and D3) were generated and compared with an unedited wildtype and a CRISPR-Sham hiPSC line. All hiPSC lines passed quality control checks including karyotyping and pluripotency (Supplementary Fig. 4b-c).
We subjected our SLC30A8-edited iPSCs to a previously published in vitro endocrine pancreas differentiation protocol\(^1\) (Supplementary Fig. 4). At the end of the seven stage protocol, SLC30A8 expression was significantly reduced in cells heterozygous for the p.Arg138* allele (clone A3 0.06±0.03, \(p<0.0001\); clone B1 0.04±0.01, \(p<0.0001\)) or homozygous for the p.Lys34Serfs50* (clone B3 0.04±0.01, \(p<0.0001\); clone D3 0.04±0.01, \(p<0.0001\)) compared to unedited control cells (1.02±0.13) (Fig. 4a). In addition, ZnT8 protein was absent in homozygous p.Lys34Serfs50* hiPSC-derived beta-like cells compared to wildtype controls, but was also undetectable in differentiated heterozygous p.Arg138* clones (Fig. 4b-c), suggesting an impact on differentiation. Indeed, the number of INS- and SLC30A8-transcript expressing cells were reduced in clones with premature stop codons in SLC30A8 (Supplementary Fig. 4d-g), indicating a reduced formation of beta-like cells. To disentangle effects on differentiation from those on expression, allele-specific SLC30A8 expression was quantified by digital droplet PCR\(^2\) in the heterozygous p.Arg138* lines. Of note, p.Arg138* allele-specific SLC30A8 expression was reduced compared to the WT allele (clone A3: 24.3±3.1%, \(p<0.0001\); clone B1: 22.2±1.7%, \(p<0.0001\)) (Fig. 4d). The reduced expression of the mutant SLC30A8 allele (clone A3: 18.2±1.8%; clone B1: 18.6%) compared to the wildtype allele (81.7±1.3%) was confirmed (\(p<0.0001\)) by targeted SLC30A8 mRNA sequencing (Fig. 4e and Supplementary Fig. 5c). Although not statistically significant inhibition of NMD by cycloheximide showed a trend towards a greater rescue of the p.Arg138* transcript compared to the p.Arg138Arg transcript (clone A3: 230±61% vs. 150±33%, \(p=0.28\) and clone B1:198±45% vs. 152±26% \(p=0.39\), Fig. 4f-g). Taken together, these data show that the protective p.Arg138*-SLC30A8 allele undergoes NMD, resulting in haploinsufficiency for SLC30A8.

**Impact of SLC30A8 loss in a human \(\beta\)-cell line**

Since human in vivo studies provided strong evidence for a role of the p.Arg138* on insulin secretion and proinsulin processing, we studied the impact of SLC30A8 loss using siRNA mediated knock down (KD) on both phenotypes in a well characterized human \(\beta\)-cell model EndoC-\(\beta\)H1\(^4\).
By siRNA, we achieved a 30-65% decrease in SLC30A8 mRNA levels (p≤0.003, Fig. 5a, Supplementary Fig. 7a) which resulted in reduced ZnT8 protein levels (Fig. 5b) associated with a reduction in intracellular zinc content (34%, p=0.002) (Fig. 5c). Assessment of dense core granule zinc content in SLC30A8-silenced EndoC-βH1 cells (Supplementary Fig. 6a), through the use of the cell surface attached fluorescent zinc probe ZIMIR (Supplementary Fig. 6b-c) revealed a tendency (p=0.10, Supplementary Fig. 6d), for reduced Zn$^{2+}$ release, consistent with the loss of zinc from this compartment, and also with previous findings in islets from Slc30a8 null mice.

KD of SLC30A8 had no significant effect on stimulated insulin secretion neither in response to 20 mM glucose nor to the sulphonylurea tolbutamide (which closes K$_{ATP}$ channels) (Fig. 5d). However, basal insulin secretion was significantly increased in siSLC30A8 transfected cells (p=0.048) and the inhibitory effect of diazoxide, a K$_{ATP}$ channel opener, on glucose-stimulated insulin secretion, was significantly reduced (p=8×10$^{-3}$, Fig. 5d). There was no effect of SLC30A8 KD on insulin content (Fig. 5e). We then measured the resting membrane conductance ($G_m$) in siSLC30A8 transfected cells incubated with 100µM diazoxide (to activate K$_{ATP}$ channels). In control cells, $G_m$ was in agreement with previous reports, while SLC30A8 KD reduced $G_m$ by 65% (p=0.002, Fig. 5f) without altering cell size (Fig. 5g). Indeed, reduced K$_{ATP}$ channel activity is consistent with the reduced expression observed of the genes encoding the K$_{ATP}$ channel subunits, SUR1 (ABCC8) and Kir6.2 (KCNJ11) (P=0.04 and 0.06 respectively, Fig. 5h). In addition, insulin secretion elicited by the combination of elevated 50mM extracellular K$^+$ ([K$^+$]$_o$) to depolarize the cells and open voltage-gated Ca$^{2+}$ channels and 16.7 mM glucose was significantly higher after SLC30A8 KD (p=0.002, Fig. 5i). The proinsulin-insulin ratios (both total and secreted hormones) and proinsulin concentrations were decreased in siSLC30A8 transfected cells (p≤0.007 and ≤0.018 respectively, Fig. 5j-k), but no differences in either protein or mRNA levels of genes (PCSK1, PCSK2 and CPE) involved in proinsulin processing in SLC30A8 KD cells was observed (Fig. 5m-p, Supplementary
However, we did observe increased AKT phosphorylation (pAKT-473) and improved cell survival under ER stress (p=0.016 and 0.016 respectively, Fig. 5q-s) in SLC30A8 KD cells. We next examined the effects of SLC30A8 KD on gene expression in EndoC-βH1 cells by mRNA sequencing (RNA-seq) of siSLC30A8 treated and siScramble cells (n=8 vs. 8). We observed a total of 674 significantly differently expressed genes among 12,956 protein coding genes that passed the quality control filters (Supplementary Fig. 7 and Supplementary Data Set 1). RNA-seq confirmed the reduction in KCNJ11 and ABCC8 expression in SLC30A8 KD cells (p=2.6 x10^{-18} and p=2.04x10^{-9} respectively, Supplementary Fig. 7c) as seen earlier by qPCR (Fig. 5h). In addition, further genes involved in the regulation of β-cell excitability/exocytosis, including CACNA1C, were up-regulated in KD cells (Supplementary Fig. 7c). Moreover, expression of genes associated with β-cell maturation and development were also influenced by SLC30A8 KD with decreased expression of NNX6.1 and PDX1 and increased expression of SOX4, SOX6 and SOX11 (Supplementary Fig. 7b). A pathway enrichment analysis of differentially expressed genes revealed enrichment of genes involved in the WNT signaling and insulin secretion pathways (Supplementary Fig. 7 and Supplementary Data Set 1). A global gene set enrichment analysis (GSEA) of all expressed genes (N=12,956) using a gene ontology database revealed enrichment of genes involved in positive regulation of TOR signaling (Supplementary Fig. 7) in KD cells. Collectively, these data demonstrate a link between SLC30A8 expression and transcriptional networks involved in cell development, cell fate and plasma membrane polarization.

**Metabolic phenotype of mice carrying the human SLC30A8 p.Arg138***

Since neither global nor tissue specific Slc30a8 KO mouse models have recapitulated the human phenotype in carriers of the SLC30A8 p.Arg138* allele, we attempted to overcome this problem by using a mouse model carrying the human Slc30a8 p.Arg138* allele. On a standard chow diet there was no evidence of enhanced insulin secretion but when subjected to a high fat diet (HFD) the mice showed a significant increase in insulin secretion (p<0.01, Fig. 6i), and similar changes in
proinsulin/insulin (p=0.0004) and proinsulin/C-peptide ratios (p<0.0001) as seen in human carriers (Fig. 6f-g). No significant changes were seen in insulin clearance, glucose or insulin tolerance (Fig. 6h, j, k).

**Impact of p.Arg138* on protein localization and cytosolic zinc distribution in INS-1 cells**

Although we found no evidence for the presence of a truncated protein in the human p.Arg138* β-cell model (Fig. 4) as also reported for the p.Arg138* mice\(^\text{10}\), we still explored the possibility that a truncated protein could result from mRNA evading NMD. Transient overexpression of tagged ZnT8-p.Arg138* fusion protein in a rat insulinoma cell line, INS-1e, showed distinct punctate distribution patterns, consistent with localization of the truncated ZnT8 protein to secretory granules, as previously observed with the full length protein\(^\text{17}\) (Supplementary Fig. 8a-c).

Additionally, Western blot showed stable expression of truncated ZnT8 in native INS1e cells (Supplementary Fig. 8d).

To investigate the effects of a truncated ZnT8 protein on cytosolic free Zn\(^{2+}\), we used a genetically-encoded Zn\(^{2+}\) sensor eCALWY-4\(^\text{18}\). Overexpression of the truncated protein (p.Arg138*, Supplementary Fig. 8e-f) had no impact on cytosolic free Zn\(^{2+}\) when expressed in INS-1(832/13) cells (Supplementary Fig. 8g).

**Influence of common SLC30A8 variants p.Trp325Arg in primary human islets**

Although we were unable to study the effects of the rare T2D-protective alleles in primary human islets we were able to assess the impact of the p.Trp325Arg genotype on *in vitro* insulin and glucagon secretion. Islets obtained from cadaveric p.Trp325 carriers secreted more insulin (p=0.0153) than p.Arg325Arg carriers when stimulated with high glucose (16.7 mM) and depolarizing [K\(^{+}\)]\(_o\) (70 mM) in line with findings of SLC30A8 KD in EndoC-βH1 (Fig. 7a-b).

Interestingly, a trend towards increased glucose responsiveness was already observed at submaximal glucose stimulation (6 mM) (Fig. 7c). Increasing glucose from 1 mM to 6 mM
stimulated insulin secretion 2.2 \((p=0.031)\) and 2.7 \((p=0.012)\) fold in p.Arg325 and p.Trp325 carriers respectively, with no effect on insulin content (Fig. 7c-d).

As SLC30A8 is highly expressed in human alpha cells\(^1\), we also measured glucagon secretion from the same islets (Fig. 7e-f). In islets from p.Arg325Arg donors, 6 mM glucose inhibited glucagon secretion by ~50% compared to 1 mM glucose. In islets from p.Trp325Arg donors, glucagon secretion at 1 mM glucose was reduced by 50% \((p=0.033)\) compared to p.Arg325Arg donors with no effect on glucagon content (Fig. 7e-f).

Finally we explored co-expression of SLC30A8 with relevant candidate genes \((INS, GCG, \text{proinsulin processing and } K_{\text{ATP}} \text{ channel subunits})\) as well as the impact of p.Trp325Arg on their expression (Fig. 7g-j). SLC30A8 transcript levels showed strong positive correlation with expression of all candidate genes \((GCG, p\leq 1.3\times10^{-7}, PCSKI, p\leq 1.5\times10^{-7}, PCSK2, p\leq 4.6\times10^{-10}, CPE, p\leq 3.2\times10^{-6}, KCNJ11, p\leq 7.1\times10^{-7}, \text{and } ABCC8, p\leq 1.6\times10^{-11})\) except INS. The protective p.Trp325 allele showed a trend (non-significant) of decreased SLC30A8 expression \((p=0.053)\) as well as genes involved in proinsulin processing such as PCSKI \((p=0.041)\), PCSK2 \((p=0.045)\) and ABCC8 \((p=0.049)\).

Taken together, these data suggest that the common T2D-protective allele (p.Trp325) may also improve the response to a glucose challenge (Fig. 2 and Fig. 3) by enhancing insulin secretion and possibly by reducing glucagon secretion in primary human islets.

**Discussion**

The current study demonstrates the strengths of using human models for studying the consequences of variants associated with human diseases. Although over 30 T2D protective LoF or missense alleles in SLC30A8 have been reported\(^9\), previous studies in rodents \(^6,17,20,21,22\), have failed to provide a mechanistic explanation for the protection. This human study robustly shows that enhanced insulin responsiveness to glucose, combined with enhanced proinsulin processing,
contribute to the protection from T2D. As all LoF carriers had the common risk p.Arg325 allele on the same haplotype, the effect of the LoF allele was most evident when compared against p.Arg325 carriers emphasizing the importance of considering the genetic background of human LoF carriers.

In our human iPSC derived beta-like cells, two different LoF alleles (p.Arg138* and p.Lys34Serfs50*) show a clear reduction in SLC30A8 expression, suggesting that NMD induced haploinsufficiency is likely a common mechanism for rare LoF alleles in SLC30A8. Although a recent study showed increased zinc transporter activity for the common risk variant p.Arg325, it should be kept in mind that the common p.Trp325Arg locus is complex as another 3’ UTR variant (rs3802177) is in strong linkage disequilibrium with p.Trp325Arg. This variant (rs3802177) may potentially affect SLC30A8 transcription/translation, further modulating the effect of p.Trp325Arg on ZnT8 function.

Interestingly, in our human iPSC based model of SLC30A8 LoF mutations (p.Arg138* and p.Lys34Serfs50*), loss of ZnT8 protein decreased the formation of INS+ cells. In support of this, we observed decreased expression of multiple genes associated with beta cell development (including PDX1) and changes in expression of genes involved in WNT signaling (including TCF7L2) after partial ZnT8 loss (KD) in the human EndoC-βH1 cell line. However, insulin content was not affected suggesting that partial ZnT8 loss is unlikely to affect beta-cell development. In support of this, complete ZnT8 null mice were viable with preserved beta-cell function. We have not been able to examine this in humans as we did find any human homozygous for SLC30A8 LoF alleles.

Whilst data from all our sub-studies are consistent with increased insulin secretion, the precise molecular mechanisms for the involvement of zinc and zinc transporters remain elusive.

Data derived from the human β-cell line show a clear decrease in intracellular zinc content and a trend towards a reduction in co-secreted granular zinc after ZnT8 loss. These observations are consistent with previous rodent studies demonstrating that loss of ZnT8 function reduces total islet
zinc content (including R138* mice) as well as free Zn$^{2+}$ in the cytosol and granules$^{6,9,17,24}$. The LoF p.Arg138* and p.Lys34Serfs50* alleles in humans are likely to exert similar effects on intracellular and granular zinc concentrations. In the present study over-expression of the truncated p.Arg138* protein in INS-1 cells did not result in changes in cytosolic zinc concentrations which is consistent with haploinsufficiency. In contrast, a recent human study showed that the T2D risk p.Arg325 allele was associated with higher islet zinc concentrations$^{25}$. In support of a potential role for zinc in the development of diabetes, Zn$^{2+}$ plays an important role as regulator of cellular excitability$^{26}$ in the CNS. In beta-cells Zn$^{2+}$ induced inhibition of L-type voltage-gated Ca$^{2+}$ channels could result in inhibition of insulin secretion$^{27}$, whilst, Zn$^{2+}$ has been reported to directly activate K$_{ATP}$ channel currents$^{28}$. The down regulation of the K$_{ATP}$ channel subunits genes following SLC30A8 knockdown suggests that there is coordinated expression of these genes. It is therefore tempting to hypothesize that SLC30A8 expression may contribute to the normal K$_{ATP}$ channel density in both $\beta$ and $\alpha$ cells but this remains to be demonstrated. The combined consequence of a reduction in K$_{ATP}$ channel gene expression and a reduction in para/autocrine electrophysiological regulation (on calcium and K$_{ATP}$ channels activities) by Zn$^{2+}$ could contribute to enhanced insulin secretion as well as reducing glucagon secretion at low glucose levels. Indeed, a similar effect on alpha cells secretory capacity has been observed upon inhibition of K$_{ATP}$ channel activity using tolbutamide$^{29}$.

Zn$^{2+}$ is essential for proper insulin crystallization, and loss of ZnT8 results in reduced dense core granules and increased granule diameter possibly due to more non-crystalline insulin which is expected to be released rapidly during exocytosis$^{6,17,30}$. The increased basal insulin secretion seen in the human $\beta$-cell line following SLC30A8 KDs and also reported in islets from Slc30a8 null mice$^{17}$ who also display impaired insulin crystallization, suggests that there is preferential release of non-crystalline insulin. It has also been suggested that increased insulin clearance could explain the decrease in circulating insulin concentrations$^{20}$. However, we did not find any support for changes
in insulin clearance in human p.Arg138* carriers or p.Arg138* mice on HFD, nor did we see any
effect on the incretin hormone GLP-1 in humans.

In contrast to the consistent effect of human p.Arg138* and p.Trp325Arg on proinsulin processing,
studies in mice following the loss of ZnT8 have reported inconsistent effects on proinsulin
processing, ranging from inhibitory effects\textsuperscript{20,21} to beneficial effects in transgenic Trp325Arg mice
on HFD\textsuperscript{22}. The reasons for this remain unclear but since blocking of the insulin receptor prevents
the positive effect on insulin processing in R138* mice\textsuperscript{10}, it is possible that insulin receptor
signaling modulates the effect of loss of ZnT8 on proinsulin processing.

The most reproducible finding in all sub-studies of SLC30A8 loss was enhanced glucose-stimulated
insulin secretion associated with increased conversion of proinsulin to C-peptide and insulin.

Carriers of the p.Trp325 T2D protective allele also showed a similar phenotype consistent with
previous published studies reporting impaired proinsulin conversion in carriers of the risk p.Arg325
allele\textsuperscript{31,32}. It has been suggested that it takes some time for insulin to mature and become
biologically active\textsuperscript{33,34}. It is therefore possible that the pronounced effect of the LoF T2D-
protective allele at 20 and 40 minutes of the test meal could reflect an effect on the time-course of
insulin maturation.

Our study has both strengths and limitations. Among its strengths are the comprehensive studies in
humans recalled by their genotype in a region of the world where the p.Arg138* allele is enriched.
The complementary studies in both human cell lines as well as the humanized mice can also be
considered as a strength. A limitation is the inconclusive measurements of zinc in different cellular
compartments and characterization of insulin granule cargo. This part will require the development
of better intracellular sensors of zinc which will enable these studies in the future.

In conclusion, our data consistently demonstrate that heterozygosity for a LoF allele p.Arg138* and
homozygosity for a common allele p.Trp325Trp of SLC30A8 are associated with increased insulin
secretion capacity and a lower risk of developing T2D and in the absence of any on-target adverse
Therefore, ZnT8 remains an appealing safe target for antidiabetic therapies preserving β-cell function.

**Online Methods**

**Human study population**

The Botnia Study has been recruiting patients with T2D and their family members in the area of five primary health care centers in western Finland since 1990. Individuals without diabetes at baseline (relatives or spouses of patients with T2D) have been invited for follow-up examinations every 3-5 years\(^1\). The Prevalence, Prediction and Prevention of diabetes (PPP)–Botnia Study is a population-based study in the same region including a random sample of 5,208 individuals aged 18 to 75 years from the population registry\(^3\). Diabetes Registry Vaasa (DIREVA) is regional diabetes registry of > 5000 diabetic patients from Western Finland (Botnia region)\(^\text{39}\). In the current study, we included >14,000 individuals (Botnia family study=5678, PPP=4862, and DIREVA=3835). All participants gave their written informed consent and the study protocol was approved by the Ethics Committee of Helsinki University Hospital, Finland (the Botnia studies) and the Ethics Committee of Turku University Hospital (DIREVA).

**Oral Glucose Tolerance Test (OGTT, Fig. 3) and test meal (Fig. 2 and Supplementary Fig. 2):**

Subjects maintained a weight-maintaining diet and avoided vigorous exercise for 3 days prior to the OGTT or test meal, which were performed after an overnight fast. Height, weight, hip and waist circumferences, fat percentage (\%, bioimpedance analyzer) and blood pressure (sitting, 3 measurements after 5 minutes rest) were measured. The participants ingested 75 g dextrose (in a couple of minutes, OGTT) or a 526 kcal mixed meal (in 10 minutes, test-meal: 76 g carbohydrate, 17 g protein and 15 g fat). Blood samples were drawn from an antecubital vein for plasma (P-) glucose and serum (S-) insulin and C-peptide at 0, 30, 120 minutes during the OGTT; for P-glucose, P-glucagon, S- insulin, S-C-peptide, S-zinc, and total S-GLP-1 at 0, 20, 40, 70, 100, 130, 160 and
190 minutes during the test meal. Test meal samples for S-FFA were collected at 0, 40 and 120 minutes and for S-proinsulin at 0, 20, 40 and 130 minutes, respectively. Urine was collected between 0–70 and 70–190 minutes for the determination of glucose and zinc excretion during the test meal.

**Intravenous Glucose Tolerance Test (IVGTT, Supplementary Fig. 3):** IVGTT group consists of total 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 Supplementary 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 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).

Corrected insulin response (CIR) was calculated for test meal (at 20 minutes) and OGTT (at 30 minutes) using the formula CIR(t)= \frac{\text{Ins}(t) \times 100}{\text{Gluc}(t) \times (\text{Gluc}(t)-3.89)}], where \text{Ins}(t) and \text{Gluc}(t) are insulin (in mU/L) and glucose concentrations (in mmol/L) at sample time point (minutes). Estimation of Insulin clearance index was done on the model based estimation of glucose-, insulin- and C-peptide curves during the test meal using the equation \frac{\text{AUC}_{ISR}}{\text{AUC}_{ins} + (I_{\text{final}} - I_{\text{basal}}) \times \text{MRT}_{\text{ins}}}, where \text{AUC}_{ISR} is the area under the curve of insulin secretion rate, \text{AUC}_{ins} is the area under the curve of insulin concentration, I_{\text{final}} is insulin concentration at the end, and I_{\text{basal}} insulin concentration at the beginning of the study. MRT_{\text{ins}} is the mean residence time of insulin, and was assumed to be 27 minutes as reported previously.

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 and imputing using the GoT2D reference panel by IMPUTEv2.

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.

Genetic Association Analysis: All the quantitative traits were inversely normally transformed before the analyses. The family-based recall study included only non-diabetic subjects during test meal. Association analysis of rare p.Arg138* with glycemic indices obtained during family-based recall test meal study (left and middle panel of Fig. 2, Supplementary Fig. 2 and Supplementary Table 4) was performed using family-based association analyses (orthogonal model together with 100,000 Monte-Carlo permutations) adjusting for age, sex, BMI, and additionally other covariates (genotype of p.Trp325Arg for middle panel only) as implemented in QTDT (v2.6.1). Association analysis of common p.Trp325Arg with glycemic indices obtained during family-based recall test meal study (right panel of Fig. 2, Supplementary Fig. 2, and Supplementary Table 4) was performed using QFAM family-based association test with 100,000 permutations to correct for any family structure as implemented in PLINK (qfam-total, mperm 100,000). The association analysis of p.Arg138* and p.Trp325Arg with glycemic traits during OGTT studies including only non-diabetic individuals (Fig. 2, Supplementary Table 5 and Supplementary Table 6) and IVGTT (Supplementary Fig. 3) was performed using mixed linear model considering genetic relatedness among samples as implemented in GCTA (v1.91). The fixed-effects meta-analysis of all OGTT studies (Supplementary Table 6) were performed using METAL software package. The linear mixed model (adjusting for genetic relatedness) also used for T2D association analysis.
Study participants and their clinical measurements in the Verona Newly Diagnosed Diabetes

Study (VNDS, Supplementary 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. 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. 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. The analysis of the glucose and C-peptide curves during the OGTT was carried out with a mathematical model as described previously. 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 and Supplementary Fig. 4 and 5)**

**iPSC generation and maintenance**: The human induced pluripotent stem cell line (hiPSC) SB Ad3.1 was previously generated and obtained through the IMI/EU sponsored StemBANCC consortium via the Human Biomaterials Resource Centre, University of Birmingham (http://www.birmingham.ac.uk/facilities/hbrc). Human skin fibroblasts were obtained from a commercial source (Lonza CC-2511, tissue acquisition number 23447). They had been collected from a Caucasian donor with no reported diabetes with fully informed consent and with ethical approval from the National Research Ethics Service South Central Hampshire research ethics committee (REC 13/SC/0179). The fibroblasts were reprogrammed to pluripotency as previously described and were subjected to the following quality control checks: SNP-array testing via Human CytoSNP-12v2.1 beadchip (Illumina #WG-320-2101), DAPI-stained metaphase counting and mFISH, flow cytometry for pluripotency markers (BD Biosciences #560589 and 560126), and mycoplasma testing (Lonza #LT07-118).

**CRISPR-Cas9 mediated generation of p.Arg138* and p.Lys34Serfs50* human induced pluripotent stem cell line**: Several guide RNAs (gRNAs) were designed using MIT CRISPR tool (http://crispr.mit.edu/) to target near exon 2 and exon 3 of SLC30A8 (ENST00000456015). The gRNAs were also subjected to an additional BlastN search (www.ensembl.org) to confirm specificity and identified no additional off-target sites. To generate SLC30A8-p.Arg138*, the target site for CRISPR-Cas9 mutagenesis (AGCAGGTACGGTTCATAGAG) was sub-cloned into the Bsbl restriction sites within the gRNA structure in the pX330 plasmid that was previously modified to contain a puromycin selection cassette. A single strand oligonucleotide repair template for homology-directed repair (HDR) was synthesized by Eurogentec, stabilized by addition of a phosphorothioate linkage at the 5’ end, and contained two nucleotide changes: i) the T2D-protective
nonsense mutation at codon-138 (c.412C>T, p.Arg138*), which also mutated the PAM sequence, and ii) a silent mutation at codon-139 (c.417A>T, p.Ala139Ala) to introduce an AluI restriction site for genotyping. Human iPSCs were co-transfected with the SLC30A8-px330-puromycin resistant vectors and the HDR repair template using Fugene6 according to manufacturer’s guidelines (Promega #E2691). Following transient puromycin-selection, single clones were picked and expanded as described previously51. Genotyping PCR was performed using primers (Forward: TACCCCCAGGAATGGCTTCTC; Reverse: ACGTGTTCCTGTTGTCCCA) to amplify targeted region followed by AluI restriction digest. Successfully targeted clones were confirmed via Sanger sequence and monoallelic sequencing was performed by TA-cloning (pGEM®-T Easy Vector System; Promega #A1360) of the PCR amplicons. From 96 clones, 11 clones were heterozygous for p.Arg138*, four of which contained indels in the non-targeted allele. The CRISPR-Sham hiPSC control line (p.Arg138Arg) was generated from hiPSC cells that went through the CRISPR pipeline without being edited at the SLC30A8 locus. The two p.Arg138* clones (A3 and B1) and the unedited control line (p.Arg138Arg) passed quality control checks that included repeat chromosome counting and pluripotency testing. Both p.Arg138* clones were heterozygous for the c.412C>T, p.Arg138*) while the silent variant at codon-139 (c.417A>T, p.Ala139Ala) was present in both alleles.

To generate SLC30A8-p.Lys34Serfs50* the gRNA addressing the target site for CRISPR/Cas9 mutagenesis (GTGAATAAAGATCAGTGTCC) 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 (Forward: TGGTGGCATTGACTGAATAAGA, Reverse: ACCCTCCCATAATGATGCAGA, and HDR-specific: GAAACCGGTGAATAGTGTCCCA) 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:** Directed differentiation of hiPSCs towards beta-like cells was performed using a previously published protocol. hiPSCs were seeded on Growth Factor Reduced Matrigel-coated CellBind 12-well tissue culture plates (Corning #356230 & #3336) at a cell density of 1.3x10^6 in mTesR1 (Stem Cell Technologies #05850) with 10 μM Y-27632 dihydrochloride (Abcam #ab120129). The following morning, hiPSCs were fed mTesR1 media >4 hours before starting the seven-stage differentiation protocol.

**Stage 1 (Definitive Endoderm):** 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.
Quantification of SLC30A8 gene expression in Beta-like Cells derived from CRISPR-edited hiPSCs:

Expression of SLC30A8 was measured at the end of stage 7 using quantitative PCR (qPCR). Briefly, RNA was extracted using TRIzol Reagent (Life Technologies #15596026) according to manufacturer’s instructions. cDNA was amplified using the GoScript Reverse Transcription Kit (Promega #A5000). qPCR was performed using 40 ng of cDNA, TaqMan® Gene Expression Master Mix (Applied Biosystems #4369017) and primer/probes for SLC30A8 (Hs00545182_m1) or the housekeeping gene TBP (Hs00427620_m1). Gene expression was determined using the ΔΔCT method by first normalizing to TBP and then to the control p.Arg138*Arg (n=7-13 wells from three differentiations).

Allele-specific SLC30A8 expression in beta-like Cells derived from CRISPR-edited hiPSCs:

Stage 7 cells were treated with 100 μg/mL cycloheximide (Sigma #C4859) or DMSO (Sigma #D2650) for four hours at 37°C before RNA and cDNA synthesis as above. Allele specific expression was measured using the QX10 Droplet Digital PCR System and C1000 Touch Thermal Cycler according to manufacturer’s guidelines (Bio-Rad). Custom primers and probes for the detection of p.Arg138* variant were designed using Primer3Plus (Applied Biosystems): Forward primer AGTCTCTTCTCCCTGTGGTT; Reverse primer ATGATCATCACAGTCGCCTG; FAM probe (R138; CT) 5’-FAM-ATGGCAGCGAGCTGA-MGB-3’; VIC probe (X138; TT) 5’-VIC-ATGGCAGCTGAGA-MGB-3’. The specificity of the probes was confirmed by ddPCR using R138 or X138 templates (Supplementary Fig. 5). Results were analysed using Quanta Soft software (Bio-Rad) and presented as a ratio of wildtype to HDR-edited allele expression (n>4 wells from two differentiations).

Allele-specific SLC30A8 expression by targeted RNA sequencing of SLC30A8 (Figure 4e and Supplementary 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, 5'-AGTCTCTTCTCCCCCTGTGGTT-3', and 5'-ATGATCATCACAGTCGCCG-3', 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)^54, 55 and UMI-tools^56 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® (Supplementary Fig. 5e-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:** We used highly specific antibody for ZnT8 as developed by Merriman et al.\(^{57}\). 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 15 minutes. 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)**

The results obtained in EndoC-βH1 are from two distinct teams (Helsinki and Oxford) with different batches of EndoC-βH1 cultures. Here, we report both methods and specify for each experiment the origin of the culture (Helsinki or Oxford). EndoC-βH1 cells were cultured in medium and grown on a matrix as described previously\(^{58}\) and tested negative for mycoplasma.
**SLC30A8 knockdown in EndoC-βH1 cells:** In Oxford, EndoC-βH1 cells were transfected with 10 nM siRNA (either SMARTpool ON-TARGETplus SLC30A8 or scramble [Dharmacon #L-007529-01]) and Lipofectamine RNAiMAX (Life Technologies #13778-075) according to manufacturer’s instructions for a total of 72 hours. In Helsinki, EndoC-βH1 cells were transfected using Lipofectamin RNAiMAX (life technologies). 20nM siRNA ON-TARGET plus siRNA SMARTpool for human SLC30A8 gene (Dharmacon; L-007529-01) and ON-TARGET plus Non-targeting pool (siNT or Scramble) (Dharmacon; D-001810-10-05) were used following the protocol as described previously. Cells were harvested 96 h post-transfection for further studies.

**Insulin secretion measurements in EndoC-βH1 cells:** In Oxford, cells were subjected to static insulin secretion assays 72hrs after siRNA transfection as described previously, apart from the following modifications: cells were stimulated for 1 hr with 1 mM glucose, 20 mM glucose, 1 mM glucose + 200 μM tolbutamide, or 20 mM glucose + 500 μM diazoxide. Insulin levels were measured in both supernatants and cells using the Insulin (human) AlphaLISA Detection Kit and EnSpire Alpha Plate Reader (Perkin Elmer #AL204C and #2390-0000, respectively). Cell count per well was measured via CyQUANT Direct Cell Proliferation Assay (Thermo Fisher# C35011). Data are presented as insulin secretion normalized to percentage of insulin content from Control condition. RNA extraction, cDNA synthesis, and qRT-PCR was performed as above (SLC30A8 gene expression in CRISPR-edited hiPSCs derived beta like cell section) to determine SLC30A8 knockdown and expression of the K\textsubscript{ATP} channel genes (ABCC8 Hs01093752_m1 and KCNJ11 Hs00265026_s1; ThermoFisher Scientific). In Helsinki, EndoC-βH1 cells were transfected with 20nM siRNA and Scramble control. Following 96h of siRNA transfection, cells were incubated overnight in 1 mM glucose containing EndoC-βH1 culture medium. One hour prior to glucose stimulation assay, the media was replaced by βKREBS (Univercell Biosolution S.A.S., France) without glucose. Cells were stimulated with 16.7 mM glucose and 50 mM KCl (Sigma-Aldrich) in βKREBS for 30 minutes at 37°C in a CO\textsubscript{2} incubator. The cells were then washed and lysed with...
TETG (Tris pH8, Trito X-100, Glycerol, NaCl and EGTA) solution (Univercell Biosolution S.A.S., France) for the measurement of total insulin content. Secreted and intracellular insulin were measured using a commercial human insulin Elisa kit (Mercodia AB, Uppsala, Sweden) as per manufacturer’s instructions (Helsinki).

Electrophysiological measurements in EndoC-βH1 cells (Oxford): SLC30A8 was knocked down in EndoC-βH1 as above. K\textsubscript{+ATP} channel conductance was measured in a perforated patch whole cell configuration, and patch-clamped using an EPC 10 amplifier and HEKA pulse software. KREBS extracellular solution was perfused in at 32°C and contained: 138 mM NaCl, 3.6 mM KCl, 0.5 mM MgSO\textsubscript{4}, 10 mM HEPES, 0.5 mM NaH\textsubscript{2}PO\textsubscript{4}, 5 mM NaHCO\textsubscript{3}, 1.5 mM CaCl\textsubscript{2}, 1 mM glucose and 100 μM Diazoxide (Sigma-Aldrich #D9035). The perforation of the membrane was achieved using an intra-pipette solution containing: 0.24 mg/mL amphotericin B, 128 mM K-glucosonate (Sigma #Y0000005 and G4500 respectively), 10 mM KCl, 10 mM NaCl, 1 mM MgCl\textsubscript{2}, 10 mM HEPES, pH 7.35 (KOH). Conductance data are normalized to cell size and presented as pS.pF\textsuperscript{-1}. Expression of ABCC8, KCNJ11, B2M, and TBP were measured via qPCR as above (SLC30A8 gene expression in CRISPR-edited hiPSCs derived beta like cell section).

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 (Helsinki): 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^61. 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

(Supplementary 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% O₂/5% CO₂ and adjusted to pH 7.4, containing 3 mM glucose and 5µM Zinc binding probe zinc indicator for monitoring induced exocytotic release (ZIMIR⁵). 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 (Supplementary 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)⁵⁴, genome annotations were obtained from the GENCODE (Encyclopedia of Genes and Gene Variants) v22⁵⁵.
program, and reads counting were done using featureCounts. Further downstream analysis was performed using edgR software package, low expressed (\(<-1 \text{ median log}_2 \text{ transformed counts per gene}\) genes were removed, read counts were normalized using TMM (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), 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) against gene ontology database (c5.all.v6.2.symbols.gmt) with 1000 permutations.

**Data Analyses:** Data are reported as mean (SEM). Statistical analyses were performed using Prism 6.0 (GraphPad Software). All parameters were analyzed using Mann-Whitney test or one sample t test as indicated.

**Mouse Model (Fig. 6)**

**Animals:** All procedures were conducted in compliance with protocols approved by the Regeneron Pharmaceuticals Institutional Animal Care and Use Committee. The Slec30a8\textsuperscript{Tgp.Arg138*} mouse line is made in pure C57Bl/6 background by changing nucleotide 409 from T into C in exon 3, which changes the arginine into a stop codon\textsuperscript{10}. The mutated allele has a self-deleting neomycin selection cassette flanked by loxP sites inserted at intron 3, deleting 29 bp of endogenous intron 3 sequence. Mice were housed (up to five mice per cage) in a controlled environment (12-h light/dark cycle, 22\degree C, 60–70% humidity) and fed \textit{ad libitum} with either chow (Purina Laboratory 23 Rodent Diet 5001, LabDiet) or high-fat diet (Research Diets, D12492; 60% fat by calories) starting at age of 10 to 20 weeks. All data shown are compared to their respective WT littermates.
**Glucose Tolerance Test:** Mice were fasted overnight (16 hr) followed by oral gavage of glucose (Sigma) at 2 g/kg body weight. Blood samples were obtained from the tail vein at the indicated times and glucose levels were measured using the AlphaTrak2 glucometer (Abbott). Submandibular bleeds for insulin were done at 0, 15, and 30 minutes post-injection in a separate experiment to not interfere with glucose levels.

**Hormone measurements:** Submandibular bleeds of either overnight fasted (16 hrs) animals were done in the morning. Plasma insulin or proinsulin was analyzed with the mouse insulin/proinsulin EIA (Mercodia AB, Uppsala, Sweden), and C-peptide with the mouse C-peptide EIA (ALPCO). All EIAs were performed according to the manufacturer’s instructions.

**Data Analyses for mouse studies:** Data are reported as mean (SEM). Statistical analyses were performed using Prism 8.0 (GraphPad Software). All parameters were analyzed by two-way ANOVA combined with Sidak’s multiple comparison test, or Mann Whitney test as indicated.

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

INS-1E cells 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** ([Supplementary 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 Zn²⁺ 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 NaH₂PO₄, 0.2 mM MgSO₄, 1.5 mM CaCl₂, 10 mM HEPES, 25 mM NaHCO₃) without additives, next the buffer was changed to KREBS buffer containing 50 µM N,N,N’,N’-tetraakis(2-pyridylmethyl)ethylenediamine (TPEN, Sigma) for 5 minutes, followed by perfusion with KREBS buffer containing 100 µM ZnCl₂ and 5 µM of the Zn²⁺-specific ionophore 2-mercaptopryridine 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 Zn²⁺ concentration using the following...
formula: \[ [\text{Zn}^{2+}] = K_d \cdot \left( \frac{(R - R_{\text{min}})}{(R_{\text{max}} - R)} \right) \), in which Rmin is the ratio in the Zn\(^{2+}\) depleted state, after addition of 50 \(\mu\)M TPEN, and Rmax was obtained upon Zn\(^{2+}\) saturation with 100 \(\mu\)M ZnCl\(_2\) in the presence of 5 \(\mu\)M pyrithione.

**Human Pancreatic Islets (Fig. 7)**

Experiments on primary human pancreatic islets were independently performed in two places 1) Oxford and 2) Lund university diabetes center (LUDC)

**Human pancreatic islets from Oxford:** Human pancreatic islets were isolated from deceased donors under ethical approval obtained from the human research ethics committees in Oxford (REC: 09/H0605/2, NRES committee South Central-Oxford B). All donors gave informed research consent as part of the national organ donation program. Islets were obtained from the Diabetes Research & Wellness Foundation Human Islet Isolation Facility, OCDEM, University of Oxford. All methods and protocols using human pancreatic islets were performed in accordance with the relevant guidelines and regulations in the UK (Human Tissue Authority, HTA). For *in vitro* insulin secretion, islets were pre-incubated in Krebs-Ringer buffer (KRB) containing 2 mg/mL BSA and 1 mM glucose for 1 hour at 37\(^\circ\)C, followed by 1-hour stimulation in KRB supplemented with 6mM glucose. Insulin content of the supernatant was determined by radioimmunoassay (Millipore UK Ltd, Livingstone, UK) as described previously\(^68\).

**Human pancreatic islets from LUDC:** Human pancreatic islets were obtained from the Human Tissue Laboratory (Lund University, www.exodiab.se/home) in collaboration with The Nordic Network for Clinical Islet Transplantation Program (www.nordicislets.org)\(^69,70\). All the islet donors provided their consent for donation of organs for medical research and the procedures were approved by the ethics committee at Lund University (Malmö, Sweden, permit number 2011263). Islet preparation for cadaver donors, their purity check and counting procedure have been described previously\(^71\). Static *in vitro* insulin secretion assay from 91 islets (non-diabetic individuals) was
performed as described previously\textsuperscript{71,72}. Briefly, six batches of 12 islets per donor were incubated for 1 hour at 37°C in Krebs Ringer bicarbonate (KRB) buffer in presence of 1 mM or 16.7 mM glucose. Independently, KCL based insulin secretion assay was performed by incubating them in 70 mM KCl together with 1 mM or 16.7 mM glucose in a subset of islets in different batches. Insulin concentrations in the extracts were measured using a radioimmunoassay kit (Euro-Diagnostica, Malmö, Sweden). The Association of p.Trp325Arg genotype with expression of \textit{SLC30A8} and other candidate genes was performed using RNA sequencing from islets of 139 non-diabetic individuals as described previously\textsuperscript{69,70}. 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)\textsuperscript{69,70}. RNA sequencing reads were aligned to hg19 using STAR (Spliced Transcripts Alignment to Reference)\textsuperscript{54}. Genome annotations were obtained from the GENCODE (Encyclopedia of Genes and Gene Variants) v20\textsuperscript{55} program and read counting was done using featureCounts\textsuperscript{62}. Read counts were normalized to total reads (counts per million) and additionally across-samples normalization was done using TMM method\textsuperscript{64}. Association analysis (so called eQTL) was performed on inverse normalized expression values using linear regression adjusted for age, sex and islets purity using PLINK\textsuperscript{36}.

**Statistics**

Detail information regarding statistical tests used for each sub-study has been provided in their respective method section or with figure legends.

**Data Availability**

The data that support the findings of this study are available from the corresponding author on reasonable request. Individual level data for the human study can only be obtained via the Biobank of The Institute of Health and Welfare in Finland. The raw count data for RNA sequencing are available in Supplementary Data Set 1.
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Author Contributions
M.L., L.S., T.T. and L.G. conducted the human study; E.A., O.H., A.B., O.P.D. and J.F. analyzed the genotype data; M.L., O.P.D., M.T., E.B., R.C.B, T.T. and L.G. analyzed the human data; B.H., A.G, N.L.B., S.K.T., M.v.D.B., V.C., O.P.D., T.O. and A.L.G. characterized the Human beta-cell model; N.A.J.K., F.A., N.L.B., B.C., D.M., P.K., B.D., O.P.D., A.S., M.I.M. and A.L.G. characterized the human IPS cell derived model; U.K., R.P., O.P.D., B.H., A.J.P., I.S., R.R., I.A., P.R., M.I.M. and A.L.G. characterized the human islets; S.K., D.G. and J.G. characterized the Sle30a8 Arg138* mice; D.J., J.O.L., P.C., A.T., R.C., A.M.R., J.B. and G.R. characterized the rat insulinoma cell-line; M.I.M., A.L.G., T.T. and L.G. supervised the project; O.P.D., M.L., B.H., N.A.J.K., S.K., P.R., A.L.G., T.T., and L.G. wrote the manuscript; all authors revised the manuscript.

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Competing interests

L.G. has received research funding from Pfizer Inc, Regeneron Pharmaceuticals, Eli Lilly and AstraZeneca. N.L.B. and M.vD.B are now employees of Novo Nordisk, although all experimental work was carried out under employment at the University of Oxford. ALG has received honoraria from Novo Nordisk and Merck. MIM serves on advisory panels for Pfizer, Novo Nordisk, Zoe Global; has received honoraria from Pfizer, Novo Nordisk and Eli Lilly; has stock options in Zoe Global; has received research funding from Abbvie, Astra Zeneca, Boehringer Ingelheim, Eli Lilly, Janssen, Merck, Novo Nordisk, Pfizer, Roche, Sanofi Aventis, Servier, Takeda. GAR is a consultant for Sun Pharma and has received grant funding from Servier. J.O.L. has received research funding from Pfizer Inc and Novo Nordisk A/S.
Figure legends

**Fig. 1:** A flow-chart describing the study design.

OGTT; oral glucose tolerance test, IVGTT; intravenous glucose tolerance test, GTT; glucose tolerance test

a, The study design including various model systems (left panels), methods (middle panels) and the purpose of these experiments (right panels).

b, Detailed description of the human in vivo studies, including a genotype-based recall study for p.Arg138* carriers and their relatives for metabolic studies.

**Fig. 2:** SLC30A8-p.Arg138* enhances insulin secretion and proinsulin processing during test meal.

Association of SLC30A8 p.Arg138* and p.Trp325Arg variants with a, plasma glucose b, serum insulin c, insulin/glucose ratio d, proinsulin/C-peptide ratio and e, proinsulin/insulin ratio during test meal. **Left panel:** Carriers (red, n=54) vs. non-carriers (black, n=47) of p.Arg138*.

**Middle panel:** Carriers of p.Arg138* (red, n=54) vs Arg138Arg having the common risk variant p.Arg325 (blue, n=31).

**Right panel:** Carriers of p.Trp325Trp (grey, n=16) vs. p.Arg325 (blue, n=31). Data are shown as Mean ± SEM. *P values (*p<0.05, **p<0.01) for family-based association (using QTDT\(^{35}\)) 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. #P value (#p<0.05) calculated using family-based QFAM test using 100,000 permutations as implemented in PLINK\(^{36}\).

**Fig. 3:** SLC30A8 p.Arg138* and p.Trp325 enhance insulin secretion during OGTT.

Association of SLC30A8 p.Arg138* and p.Trp325Arg with a, plasma glucose b, serum insulin c, insulin/glucose ratio during an oral glucose tolerance test (OGTT). **Left panel:** Carriers (red, n=34-35) vs. non-carriers (black, n=7954-8141) of p.Arg138*.

**Middle panel:** Carriers of p.Arg138* (red, n=34-35) vs. p.Arg138Arg having the common risk variant p.Arg325 (blue, n=6728-6893). **Right panel:** Carriers of p.Trp325Trp (grey n=1226-1248) vs. p.Arg325 (blue, n=6728-6893). Data are shown as Mean ±SEM. *P values (*p < 0.05, **p<0.01) for additive effect were calculated using mixed model adjusting for genetic relationship, age, sex, BMI and additionally genotype of p.Trp325Arg only for middle panel as implemented in GCTA\(^{37}\). Y-axis: note truncation (\(<\)) and different scale in the right panel.
Fig. 4: Beta like cells derived from SLC30A8-p.Arg138* iPSCs display haploinsufficiency of SLC30A8.

a-c, Characterization of SLC30A8 expression at the (a) mRNA and (b-c) protein level in cells heterozygous for SLC30A8-p.Arg138* or homozygous for SLC30A8-p.Lys34Serfs50*. Gene expression data normalized to TBP and expressed as fold change relative to p.Arg138Arg control (n=7-13 wells from three differentiations). d-e, Allele-specific expression (ASE) of p.Arg138Arg (black bar) and p.Arg138* (red bar) in clone A3 or clone B1 derived cells using (d) Digital Droplet PCR based probes also validated by (e) target SLC30A8 mRNA sequencing of p.Arg138* clones (n=number of unique sequencing reads for each allele). f-g, Allele-specific expression of p.Arg138Arg (black bar) and p.Arg138* (red bar) in f, clone A3 and g, clone B1 derived cells treated with DMSO (Dimethyl sulfoxide) or cycloheximide (CHX) for four hours. ASE data (Mean±SEM) determined by Digital Droplet PCR were presented as fold change relative to p.Arg138Arg transcript (d, n=5-7 wells from three differentiations) or to DMSO control (f-g, n=9 wells from two differentiations). *p<0.05, **p<0.01, ****p<0.0001 one-way ANOVA Holm-Sidak’s multiple comparison test (a) or one-sample t-test (d, f and g) or binomial test considering 0.5 as expected probability (e).

Fig. 5: SLC30A8 knock down leads to enhanced insulin secretion, proinsulin processing and cell viability in the human pancreatic EndoC-βh1 cells.
a-b, Effect of siRNA mediated knock down (KD) on SLC30A8 mRNAs and protein. c, Measurement of intracellular zinc using zinc-specific fluorescent dye Zinpyr-1. d-i, Effect of KD on (d) insulin secretion stimulated by glucose and K_ATP channel regulators (as labelled), (e) insulin content, (f) K_ATP channel conductance (Gm), (g) cell size, (h) expression of K_ATP channel subunits, (i) insulin secretion stimulated by KCL and high glucose. j-p, Effect of KD on proinsulin processing estimated by proinsulin/insulin ratio (j), proinsulin concentration (k) and protein expression of proinsulin processing enzymes PC1/3, CPE and PC2 (immunoblot- l and p, densitometry- m, n and o). q-s, Effect of KD on basal (5.5 mM glucose) AKT phosphorylation (q, densitometry, r, immunoblot; phospho-AKT-Ser473, total AKT) and cell viability under ER stress (s, MTT assay, 10 µg/ml tunicamycin, DMSO as vehicle control). Data are shown as Mean ±SEM (n=3-10). *p<0.05, **p<0.01, ***p<0.001 using Mann-Whitney test; #p<0.05 one-sample t-test; d (Bonferroni multiple correction for 4 conditions).
Fig. 6: Male p.Arg138* mice on high-fat diet show enhanced insulin secretion and proinsulin processing.

a. Body weight, circulating b. glucose c. insulin d. proinsulin (*p=0.011) e. C-peptide f. proinsulin/insulin ratio (*p=0.0004) g. proinsulin/C-peptide ratio (*p<0.0001) and h. insulin/C-peptide ratio in fasted WT and p.Arg138* mice (n= 10 WT, 17 p.Arg138*) after 20 weeks on HFD.

i. Insulin response to oral glucose (2g/kg) exposure (n=5 WT, 9 p.Arg138*) after 29 weeks on HFD and j. Blood glucose levels over time after j. oral glucose (2g/kg) exposure (n=5 WT, 11 p.Arg138*) after 29 weeks on HFD and k. interperitoneal injection of insulin (1.75 U/kg) after 28 weeks on HFD (n=11 WT, 13 p.Arg138*). *p<0.05, ***p<0.001, ****p<0.0001 using Mann Whitney test; *p<0.05, **p<0.01 using two-way Anova and Sidak’s multiple comparison test.

Fig. 7: SLC30A8- p.Trp325 leads to enhanced insulin secretion in human islets.

Experiments have been performed in two different centers: LUDC (a, b, g, h, i and j) and Oxford (c, d, e and f). a. Effect of p.Trp325Arg genotype on static insulin secretion in presence of low and high glucose stimulatory conditions. b. Effect of p.Trp325Arg genotype on static insulin secretion in presence of low or high glucose and KCL. c-d. Effect of p.Trp325Arg genotype on static insulin secretion in (c) sub-maximal stimulatory conditions (6mM glucose) and their (d) insulin contents. e. Static glucagon response to glucose and f. glucagon content at basal glucose. g-h. Correlation of SLC30A8 expression with candidate genes of INS, GCG, proinsulin processing genes and K\text{ATP} channel subunits genes among (g) p.Arg325Arg individuals and (h) p.Trp325 carriers and effect of p.Trp325Arg genotype (p.Arg325Arg=65, p.Trp325Arg=63 and p.Trp325Trp=11) on expression (cpm=log2 of counts per million) of (i) SLC30A8 and (j) other candidate genes. Data are Mean ±SEM; Glu- glucose. *p<0.05, ***p<0.0001 using Mann-Whitney test (b, c, d, e and f) or Spearman correlation coefficient (r) with two-tailed p values (g and h). Three genotype comparison (a, i and j) by linear regression using additive effect adjusting for age, sex and islet purity as implemented in PLINK.36
**Screening for p.Arg138* mutation in Botnia cohort (>14,000 individuals)**

- **Newly diagnosed T2D individuals (VNDS study)**
  - **Type 2 diabetes** (T2D) individuals (VNDS study)
  - **Non-diabetic individuals** (p.Arg138* = 55)

**Association (family based) with test meal-derived indices using glucose, insulin, C-peptide, proinsulin, glucagon, GLP1, and FFA measurements**

**Association (Meta-analysis) with OGTT-derived indices using glucose and insulin measurements**

**Association with IVGTT-derived indices using glucose and insulin measurements**

**Association with OGTT-derived indices using glucose and insulin measurements**
p.Arg325Arg individuals, n=65

p.Trp325Arg carriers, n=74

p=0.25

p=0.049

Gene expression (cpm)

p=0.041

Gene expression (cpm)

p=0.045

Gene expression (cpm)

p=0.053

p=0.041

p=0.045