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Increased expression of the diabetes gene *SOX4* reduces insulin secretion by impaired fusion pore expansion

Justification for the requirement of the following supplementary material

Supplementary Table S1.

Full list of genes showing a significant ≥ 1.5 or ≤ -1.5 fold expression change in *Sox4mt* islets.

Supplementary Figure S1.

Details how the P2X₂ current transient were analyzed and complements Figure 4D-E.

Supplementary Figure S2.

Levels of *Sox4wt* and *mt* expression achieved in a rat insulinoma cell line by transfection which serves as a control for Figure 6A.

Supplementary Figure S3.

(A-B) Focuses on expression data from human data sets and strengthens a role of SOX4 in human diabetes by supporting the previously published increased SOX4 expression in human diabetic islets (Supp. Fig. 3A) and reporting a positive correlation between *CDKAL1* and *SOX4* expression (Supp. Fig. 3B).

(C-D) characterizes the human beta cell line EndoC- β H2 and justifies the use of 20 mM G + KCl for stimulation in Figure 7E-F (Supp. Fig. 3C) and explores the expression of other exocytotic genes after *SOX4* overexpression (Supp. Fig. 3D).

Supplementary Figure S4.

Schematic explaining the relationship between STXBP6 expression and fusion pore expansion.

SUPPLEMENTARY DATA

Supplementary Table 1. Effects of *Sox4mt* on gene expression.

GCRMA (Guanine Cytosine Robust Multi-Array Analysis) normalized differentially expressed genes with $P \leq 0.05$ (Welch t-test) and a ≥ 1.5 -fold differential expression between *Sox4mt* and *Sox4wt* islets. Lists the genes with increased or reduced expression in *Sox4mt* islets. The microarray was carried out using RNA prepared from islets of 22-weeks old *Sox4mt* and *Sox4wt* mice (4 animals per genotype). Genes highlighted in red are involved in exocytosis

<i>Fold increase</i>	<i>P-value</i>	<i>Gene title</i>	<i>Gene name</i>
2.9	0.042	Gastrin	Gast
2.8	0.00311	RIKEN cDNA 6430502M16 gene	6430502M16Rik
2.4	0.0142	"deiodinase, iodothyronine, type I"	Dio1
2.4	0.0263	carboxypeptidase B2 (plasma)	Cpb2
2.2	0.0314	cell division cycle associated 8	Cdca8
2.2	0.0292	centromere autoantigen F	Cenpf
2.1	0.0304	topoisomerase (DNA) II alpha	Top2a
2.1	0.0222	apolipoprotein F	Apof
2.1	0.0207	syntaxin binding protein 6 (amisyn)	Stxbp6
2.1	0.00504	RIKEN cDNA4933405111gene-mucin 4	4933405111RikMuc4
2.0	0.0014	Expressed sequence BB164513	BB164513
1.9	0.0441	extracellular proteinase inhibitor	Expi
1.9	0.0234	antigen identified by monoclonal antibody Ki 67	Mki67 ///LOC638774
1.9	0.0438	shugoshin-like 1 (S. pombe)	Sgol1
1.9	0.0307	Sterol O-acyltransferase 1	Soat1
1.9	0.0264	cell division cycle associated 8	Cdca8
1.8	0.0212	budding uninhibited by benzimidazoles 1 homolog (S. cerevisiae)	Bub1
1.8	0.0119	regulator of G-protein signaling 16	Rgs16
1.8	0.0382	cyclin A2	Ccna2
1.8	0.0304	melanoma associated antigen (mutated) 1-like 1	Mum111
1.8	0.000652	FXYD domain-containing ion transport regulator 3	Fxyd3
1.8	0.0253	kinesin family member 20A	Kif20a
1.8	0.00912	"upstream binding transcription factor, RNA polymerase I"	Ubtf
1.8	0.0422	"Histone 1, H3i"	Hist1h3i
1.8	0.0115	aminoadipate-semialdehyde synthase	Aass

SUPPLEMENTARY DATA

1.8	0.038	regulator of G-protein signaling 16	Rgs16
1.7	0.0169	RNA binding motif protein 14	Rbm14
1.7	0.0256	mucosal vascular addressin cell adhesion molecule 1	Madcam1
1.7	0.0238	RIKEN cDNA 2810417H13 gene	2810417H13Rik
1.7	0.0322	ubiquitin-conjugating enzyme E2C	Ube2c
1.7	0.0489	Shc SH2-domain binding protein 1	Shcbp1
1.7	0.013	"angiotensinogen (serpin peptidase inhibitor, clade A, member 8)"	Agt
1.7	0.0454	thioredoxin interacting protein	Txnip
1.7	0.00135	nitric oxide synthase trafficker	Nostrin
1.7	0.0186	estrogen-related receptor gamma	Esrrg
1.7	0.00517	"malic enzyme 3, NADP(+)-dependent, mitochondrial"	Me3
1.6	0.0256	RIKEN cDNA 1110064P04 gene	1110064P04Rik
1.6	0.011	centromere protein E	Cenpe
1.6	0.0126	zinc finger and BTB domain containing 16	Zbtb16
1.6	0.0136	"DNA segment, Chr 17, human D6S56E 5"	D17H6S56E-5
1.6	0.0107	protein C	Proc
1.6	0.0389	thyroid hormone receptor interactor 13	Trip13
1.6	0.0115	RIKEN cDNA 4632411J06 gene	4632411J06Rik
1.6	0.00872	thioredoxin interacting protein	Txnip
1.6	0.035	CD44 antigen	Cd44
1.6	0.00834	"cysteine and histidine-rich domain (CHORD)-containing, zinc-binding protein 1"	Chordc1
1.6	0.00406	myosin XVb	Myo15b
1.6	0.0332	RAS-related C3 botulinum substrate 2	Rac2
1.6	0.00409	baculoviral IAP repeat-containing 5	Birc5
1.6	0.0449	RIKEN cDNA 1200016G03 gene	1200016G03Rik
1.6	0.0232	fidgetin-like 1	Fignl1
1.6	0.0348	RIKEN cDNA 1700022C02 gene	1700022C02Rik
1.5	0.00722	SMC2 structural maintenance of chromosomes 2-like 1	Smc2l1
1.5	0.00127	Mohawk	Mkx
1.5	0.0418	RIKEN cDNA 4632411J06 gene	4632411J06Rik
1.5	0.0373	relaxin/insulin-like family peptide receptor 2	Rxfp2
1.5	0.0194	RNA binding motif protein 14	Rbm14
1.5	0.0116	Hemochromatosis	Hfe
1.5	0.00648	nudix(nucleoside diphosphate linked moiety X)type motif4	Nudt4

SUPPLEMENTARY DATA

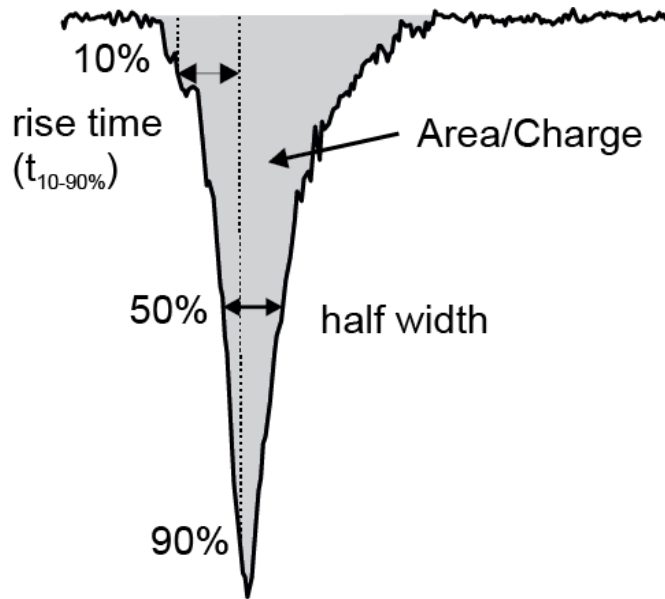
<i>Fold Decrease</i>	<i>P-value</i>	<i>Gene title</i>	<i>Gene name</i>
1.5	0.0243	thrombospondin 1 /// similar to thrombospondin 1	Thbs1 //LOC640441
3.3	0.00228	RIKEN cDNA 1100001E04 gene	1100001E04Rik
3.0	0.0208	glutamic acid decarboxylase 1	Gad1
2.7	0.0111	par6 partitioning defective 6 homolog γ (C. elegans)	Pard6g
2.7	0.0457	glutamic acid decarboxylase 1	Gad1
2.4	0.0262	cerebellin 4 precursor protein	Cbln4
2.2	0.0162	protocadherin 8	Pcdh8
2.2	0.000536	RIKEN cDNA 2210019111 gene	2210019111Rik
2.2	0.0339	aminolevulinic acid synthase 2, erythroid	Alas2
2.2	0.00775	RAB3C, member RAS oncogene family	Rab3c
2.2	0.0322	leucine-rich repeat LGI family, member 1	Lgi1
2.1	0.0177	Transcribed locus	---
2.0	0.000123	RAB3C, member RAS oncogene family	Rab3c
1.9	0.0112	nicotinamide nucleotide adenyltransferase 2	Nmnat2
1.9	0.0482	hypothetical protein A230106N23	A230106N23
1.8	0.0087	expressed sequence AI428795	AI428795
1.8	0.0185	angiopoietin-like 7	Angptl7
1.8	0.0393	RIKEN cDNA A830010M20 gene	A830010M20Rik
1.8	0.00221	double C2, beta	Doc2b
1.8	7.82E-05	RIKEN cDNA 2300002D11 gene	2300002D11Rik
1.8	0.00523	cDNA sequence AB182283	AB182283
1.8	0.0234	transient receptor potential cation channel, subfamily M, member 5	Trpm5
1.7	0.0054	polo-like kinase 3 (Drosophila)	Plk3
1.7	0.0314	eyes absent 1 homolog (Drosophila)	Eya1
1.7	0.00572	transient receptor potential cation channel, subfamily M, member 5	Trpm5
1.7	0.0383	nuclear transcription factor, X-box binding 1	Nfx1
1.7	0.0395	solute carrier family 29 (nucleoside transporters), 4	Slc29a4
1.7	0.0466	CEA-related cell adhesion molecule 10	Ceacam10
1.7	0.0438	protocadherin alpha 1/ 2/ 3/ 4/ 5/ 6/ 7/ 8/ 9/ 10/ 11/ 12/ subfamily C, 1 / subfamily C, 2	Pcdha4/ 2/ 3/ 4/ 5/ 6/ 7/ 8/ 9/ 10/ 11/ 12// Pcdhac1/ 2
1.7	0.0433	receptor accessory protein 1	Reep1
1.7	0.0359	expressed sequence AW146242	AW146242
1.7	0.00376	RAS protein-specific guanine nucleotide-releasing factor 1	Rasgrf1

SUPPLEMENTARY DATA

1.7	0.000447	glucagon receptor	Gcgr
1.6	0.0391	dual specificity phosphatase 26 (putative)	Dusp26
1.6	0.00487	F-box and leucine-rich repeat protein 10	Fbx10
1.6	0.0149	RIKEN cDNA 1500005I02 gene	1500005I02Rik
1.6	0.0092	SEC14-like 1 (<i>S. cerevisiae</i>)	Sec141
1.6	0.0142	high mobility group box 3	Hmgb3
1.6	0.00899	leucine-rich repeat LGI family, member 1	Lgi1
1.6	0.0291	cDNA sequence BC020077	BC020077
1.6	0.0156	tubulin, beta 3	Tubb3
1.6	0.0191	RAS protein-specific guanine nucleotide-releasing factor 1	Rasgrf1
1.6	0.0338	RIKEN cDNA 2310028N02 gene	2310028N02Rik
1.6	1.31E-05	latrophilin 3	Lphn3
1.6	0.0384	RIKEN cDNA 6330500D04 gene	6330500D04Rik
1.6	0.0443	RIKEN cDNA 6720475J19 gene /// similar to putative retrovirus-related gag protein	6720475J19Rik /// LOC670480
1.6	0.0339	solute carrier family 39 (metal ion transporter), 8	Slc39a8
1.6	0.0226	acyl-CoA thioesterase 7	Acot7
1.6	0.0197	Adult male corpora quadrigemina cDNA, RIKEN full-length enriched library, clone:B230347A18	---
1.6	0.0298	cDNA sequence BC039632	BC039632
1.5	0.00205	Transcribed locus, moderately similar to XP_574723.1 PREDICTED: similar to LRRGT00097	---
1.5	0.0448	Vac14 homolog (<i>S. cerevisiae</i>)	Vac14
1.5	0.0327	expressed sequence AI428795	AI428795
1.5	0.0478	expressed sequence AI428795	AI428795
1.5	0.026	ciliary neurotrophic factor /// zinc finger protein 91	Cntf /// Zfp91
1.5	0.0256	immunoglobulin superfamily, member 4A	Igsf4a
1.5	0.0385	brachyury 2	T2

SUPPLEMENTARY DATA

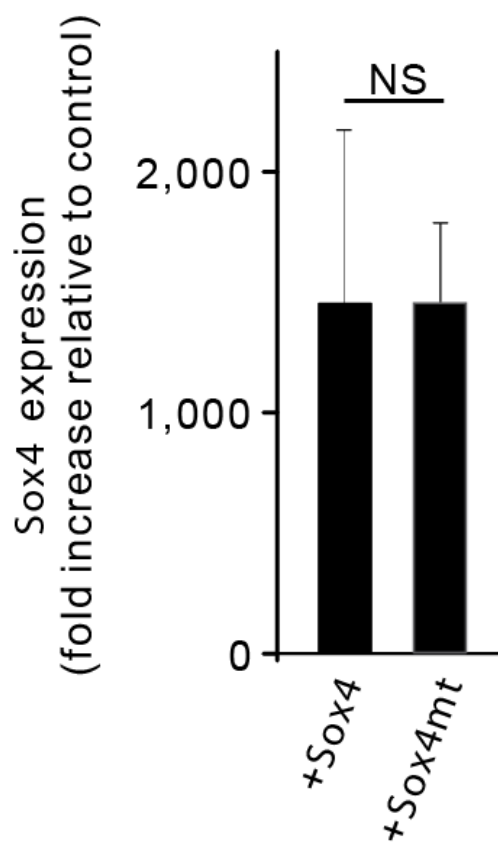
Supplementary Figure S1. Schematic of a P2X₂ current transient recorded from a *Sox4wt* β-cell expressing ionotropic P2X₂ receptors infused with 2 μM free [Ca²⁺]_i illustrating how the rise time ($t_{10-90\%}$; time required for the current to increase from 10% to 90% of the peak amplitude), the half-width (the period that the amplitude exceeds 50% of the peak) and the charge (stippled area) were determined. The dotted vertical lines indicate the traces point at 10% and 90% of the maximal amplitude.



SUPPLEMENTARY DATA

Supplementary Figure S2. Expression of *SOX4* in INS-1 832/13 cells following transfection with wild-type and mutant *Sox4*.

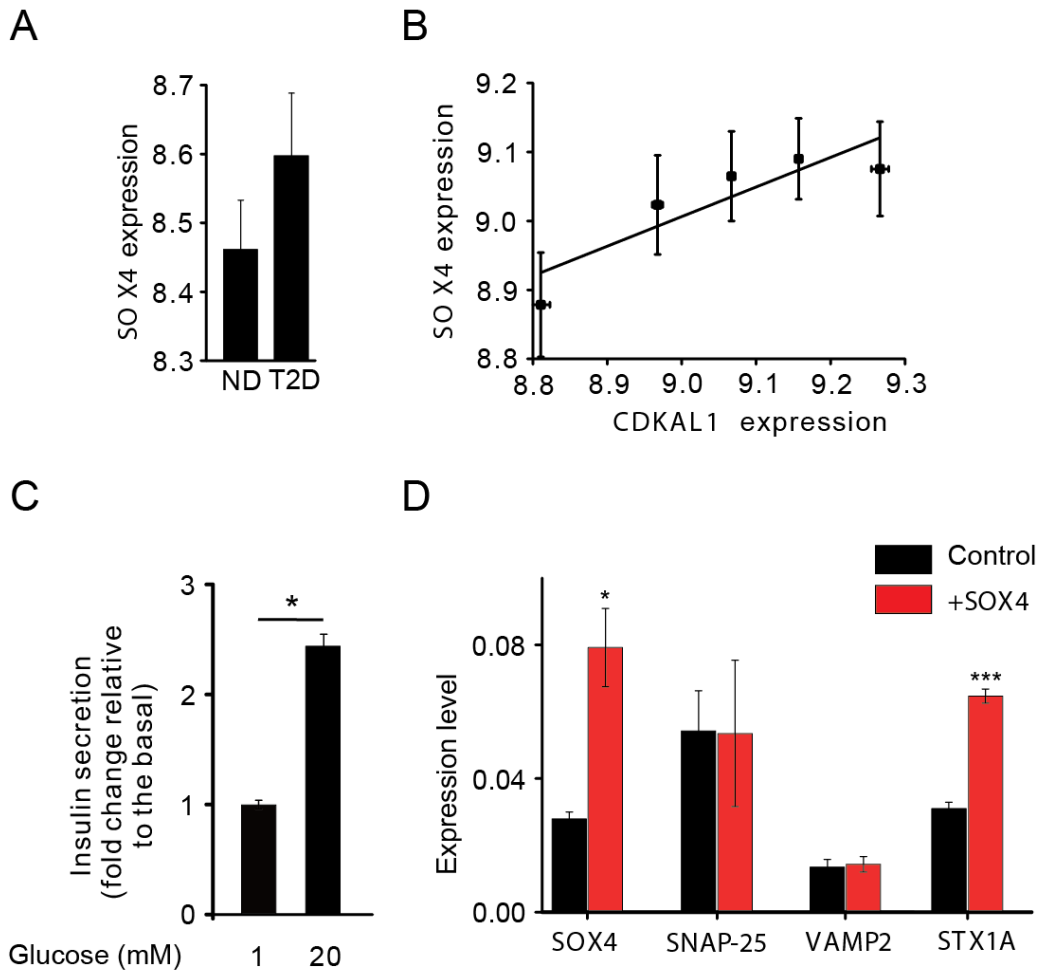
Sox4 expression was assessed in INS-1 832/13 cells transfected with scrambled siRNA + wt*Sox4* + hGH ('+*Sox4*') or scrambled siRNA + *Sox4*mt + hGH ('+*Sox4*mt'). Expression was normalised to that in cells expressing scrambled siRNA, DsRed and hGH (control).



SUPPLEMENTARY DATA

Supplementary Figure S3. Gene expression in human islets and insulin secretion in human β -cell line.

A: SOX4 expression in islets from non-diabetic (ND) and type-2 diabetic (T2D) organ donors. Values are presented as mean \pm S.E.M.; n=31 for ND donors and n=21 for T2D donors. *B:* Relationship between CDKAL1 and SOX4 expression. R=0.191; P=0.036. For display, data have been grouped in quintiles. Best-fit black line represents Pearson correlation analysis to the individual data points. *C:* Insulin secretion in untransfected EndoC- β H2 cells exposed to 1 and 20 mM glucose. Mean \pm S.E.M. of 3 experiments. *P<0.05 for the indicated comparisons. *D:* qRT PCR expression of exocytotic proteins (SNAP25, VAMP2 and STX1A (syntaxin-1A)) in control (black; scrambled siRNA + DsRed + hGH) and SOX4-overexpressing (red; scrambled siRNA + wtSOX4 + hGH) EndoC- β H2 cells. Data are presented as mean \pm S.E.M. of 3 experiments, each with 3 replicates; *P<0.01 and ***P<0.001 for the indicated comparisons.



SUPPLEMENTARY DATA

Supplementary Figure S4.

Schematic explaining the relationship between *STXBP6* expression, fusion pore opening and ATP/insulin release. When *STXBP6* is expressed at low levels (i.e. *Sox4*wt cells; bottom), full fusion (top) associated with the rapid release of both ATP and insulin is the predominant form of exocytosis, giving rise to sharp spikes of ATP release detected by the Pxx2R-based assay (middle).

A moderate increase in *STXBP6* expression (as seen in *Sox4*mt mice) stabilizes the fusion pore and delays/prevents its full opening in 50% of cases. Insulin is too bulky to be released via the fusion pore and its release depends on the eventual expansion of the fusion pore (if it occurs at all). However, ATP is small enough to exit via the fusion pore, giving rise to elongated spikes.

Strong overexpression of *STXBP6* (as exemplified in the EndoC β H2 cells transfected with *STXBP6*) prevents fusion pore expansion and for the granules affected, the fusion pore will only partially and transiently open, giving rise to short-lived bouts of ATP release, before returning to the closed state. Under these conditions, insulin will not be released, explaining the strong suppression of hormone release observed.

