

SUPPLEMENTAL MATERIAL

Syntaxin 4 enrichment in β -cells prevents conversion to autoimmune diabetes in non-obese diabetic (NOD) mice

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Supplemental Table 1. Human donor information for samples used in Fig.1A.

nPOD Case ID (islet number imaged)	Donor status	Age (years)	BMI	HbA1c	Duration of T1D (years)	C-peptide level
6271 (2)	Non-T1D	17	24.4	NA	NA	11.47
6420 (2)	Non-T1D	11.5	15.4	5.2	NA	1.27
6391 (2)	Non-T1D	10.9	19	5.3	NA	4.23
6227 (2)	Non-T1D	17	26.4	NA	NA	2.75
6480 (3)	T1D	17.2	27.1	10.2	2.5	0.13
6380 (3)	T1D	11.6	14.6	13.5	0	0.22
6268 (2)	T1D	12	26.6	9.8	3	0.05

BMI, body mass index; *HbA1c*, glycated hemoglobin; *nPOD*, Network for Pancreatic Organ Donors with Diabetes; *T1D*, type 1 diabetes.

Supplemental Table 2. Human donor information for islets used in Fig 1B.

UNOS/RRID	Age	Gender	BMI	HbA1c	Diabetes	Experimental use
ADDV480*	52y	M	25.4	5.5	No	Cytokine treatment
SAMN08769189	49y	M	29.0	5.1	No	Cytokine treatment
SAMN10869303	25y	M	27.7	5.2	No	Cytokine treatment
SAMN10977276	52y	M	27.0	5.7	No	Cytokine treatment

*UNOS ID. *BMI*, body mass index; *HbA1c*, glycated hemoglobin; *RRID*, Research Resource Identifier; *T1D*, type 1 diabetes; *UNOS*, United Network for Organ Sharing.

Supplemental Table 3. Primer sequences used for quantitative real-time PCR.

Species	Gene	Forward primer (5' to 3')	Reverse primer (5' to 3')
Rat	CXCL10	GCAAGTCTATCCTGTCCGCAT	GGGTAAAGGGAGGTGGAGAGA
	STX4	TTGGAGAAACAGCAGGTCACC	AGGCCCTGCTTCATGCTCT
	GAPDH	AGTTCAACGGCACAGTCAAG	TACTCAGCACCCAGCATCACC
	CCL5	ACACCACTCCCTGCTGCTTT	CATCTCCAATAGTTGATGTATTCTTGAAC

Supplemental Table 4. Body and tissue weights of female NOD-i β STX4 and NOD-Ctrl mice.

	NOD-Ctrl (RIP-rtTA/+;+/+) With Dox treatment Mean \pm SE	NOD-i β STX4 (RIP-rtTA/+;TRE-STX4/+) With Dox treatment Mean \pm SE
BW (g)	21.98 \pm 0.37	20.73 \pm 0.51
Tissue weight (% of body weight)		
Liver (% of BW)	3.92 \pm 0.19	4.13 \pm 0.19
Heart (% of BW)	0.61 \pm 0.10	0.74 \pm 0.08
Fat (% of BW)	1.18 \pm 0.17	0.85 \pm 0.19
Skeletal Muscle (% of BW)	2.07 \pm 0.15	2.40 \pm 0.17
Kidney (% of BW)	1.21 \pm 0.14	1.26 \pm 0.07
Spleen (% of BW)	0.18 \pm 0.03	0.23 \pm 0.04
Lung (% of BW)	0.88 \pm 0.07	0.71 \pm 0.04
Pancreas (% of BW)	0.99 \pm 0.13	1.24 \pm 0.16

BW, body weight. Littermate female NOD-i β STX4 and NOD-Ctrl mice aged 13 weeks, 4-5 mice per group (Skeletal muscle: $p=0.19$, Fat: $p=0.24$, *not significant*).

Supplemental Information

Immunofluorescence analysis of mouse pancreas: Paraformaldehyde-fixed paraffin embedded mouse pancreatic sections were immunostained with guinea pig anti-insulin (DAKO/Agilent, Santa Clara, CA; Cat# A0564), mouse anti-glucagon (Sigma, St. Louis, MO, Cat# G2654) and rabbit anti-STX4 (Chemicon/Millipore, Temecula, CA, Cat# AB5330) antibodies. We used the Alexa Fluor 488-conjugated goat anti-guinea pig (Thermo Scientific, Waltham MA, Cat# A-11073), Alexa Fluor 568 conjugated goat anti-rabbit (Thermo Scientific, Cat# A-11011), and Alexa Fluor 647-conjugated goat anti-mouse (Thermo Scientific, Cat# A-21235) secondary antibodies (Invitrogen, Carlsbad, CA). Sections were scanned with a Keyence fluorescence microscope (Itasca, IL).

Mouse tissue immunoblots: Hypothalamus and cerebellum from mouse brain were isolated and using a method described previously (1). STX4 antibody was generated for use in immunoblotting, as previously described (1) and Tubulin was used as loading control (Sigma-Aldrich, St. Louis, MO, Cat #T5168).

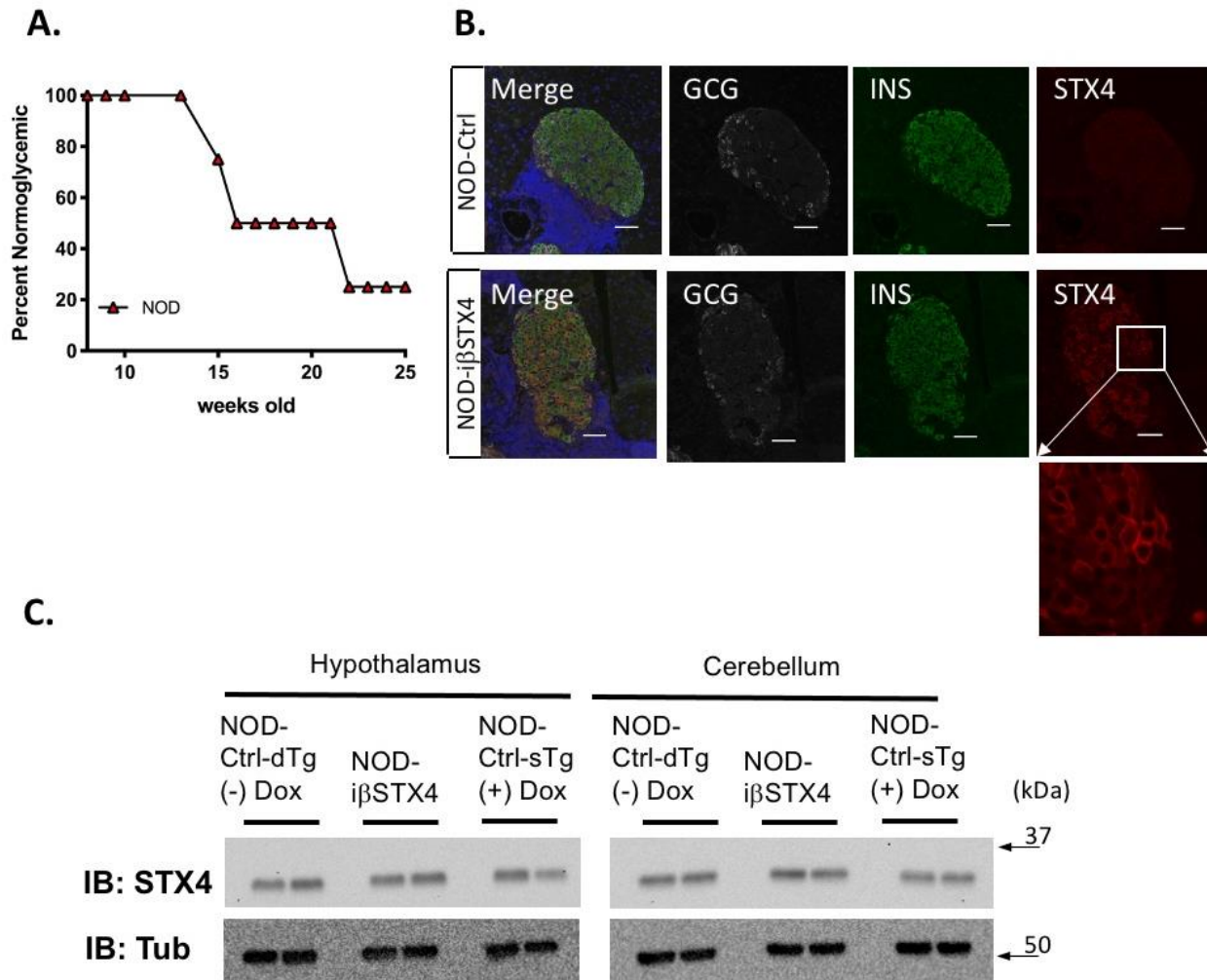
Plasma insulin measurement and Insulinitis: For plasma insulin response to an acute glucose challenge, female mice were fasted for 6 h prior to an acute challenge of 2 g glucose/kg body weight injected intraperitoneally. To measure the plasma insulin levels, blood was collected from the tail vein prior to and 10 min after the glucose injection, and the plasma was analyzed using a sensitive rat insulin radioimmunoassay kit (Millipore, cat. # SRI-13K). For histological scoring of insulinitis, paraformaldehyde-fixed and paraffin embedded whole pancreas slides were stained with hematoxylin and eosin. The score was given for each mouse based on evaluation of all islets identified by morphology using the standard scale (2,3): 0= no infiltrate, 1=peri-infiltrate only, 2=infiltrating cells extending peri islet <50%, 3=invasive insulinitis >50%, as described (4).

INS-1 cell culture and CXCL10 mRNA analysis: Cells from the INS-1 β -cell line were cultured as previously described (1), adenovirally transduced (multiplicity of infection=100) for 2 h, washed with PBS, and incubated for 48 h. For knockdown of STX4, siRNAs were purchased from Qiagen (Valencia, CA, as shown in Suppl. Table 3) and were transfected (50 nM) with RNAiMAX (Invitrogen). For the last 16 h of incubation, the cells were exposed to medium containing proinflammatory cytokines, as previously described (1) and RNA was isolated for quantitative real-time PCR.

References

1. Oh E, Ahn M, Afelik S, Becker TC, Roep BO, Thurmond DC. Syntaxin 4 Expression in Pancreatic β -Cells Promotes Islet Function and Protects Functional β -Cell Mass. *Diabetes* 2018; 67:2626-2639
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3. Simon G, Parker M, Ramiya V, Wasserfall C, Huang Y, Bresson D, Schwartz RF, Campbell-Thompson M, Tenace L, Brusko T, Xue S, Scaria A, Lukason M, Eisenbeis S, Williams J, Clare-Salzler M, Schatz D, Kaplan B, Von Herrath M, Womer K, Atkinson MA. Murine antithymocyte globulin therapy alters disease progression in NOD mice by a time-dependent induction of immunoregulation. *Diabetes* 2008; 57:405-414
4. Parker MJ, Xue S, Alexander JJ, Wasserfall CH, Campbell-Thompson ML, Battaglia M, Gregori S, Mathews CE, Song S, Troutt M, Eisenbeis S, Williams J, Schatz DA, Haller MJ, Atkinson MA. Immune depletion with cellular mobilization imparts immunoregulation and reverses autoimmune diabetes in nonobese diabetic mice. *Diabetes* 2009; 58:2277-2284

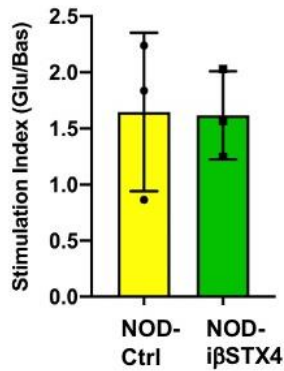
Fig. S1



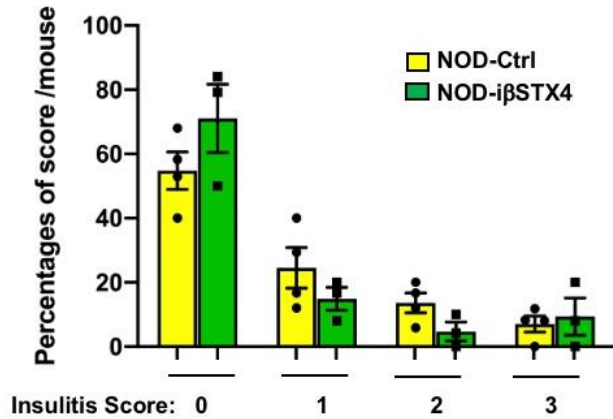
Supplemental Figure 1. Validation of NOD mouse conversion to diabetes and β -cell-specific STX4 overexpression in mouse islets. (A) Conversion rate of NOD mice in the City of Hope colony originating from JAX labs. **(B)** Immunofluorescence colocalization of STX4 with insulin (INS, β -cells) but not glucagon (GCG, α -cells), Scale bar= 50 μ m. **(C)** STX4 protein (35 kDa) levels in hypothalamus and cerebellum from mice with/without Doxycycline (Dox) treatment (1 mg/ml in drinking water for 8 weeks). No differences were observed among the treatment groups IB: immunoblot. Tub: Tubulin. n=3 mice/group.

Fig. S2

A. Plasma insulin



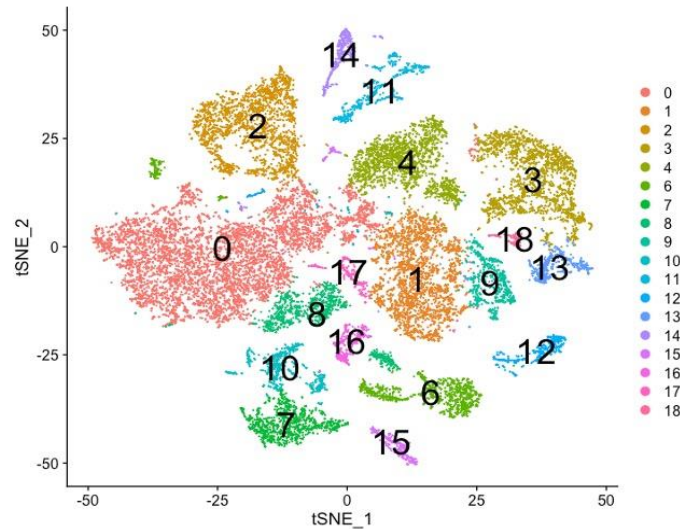
B. Insulinitis scoring



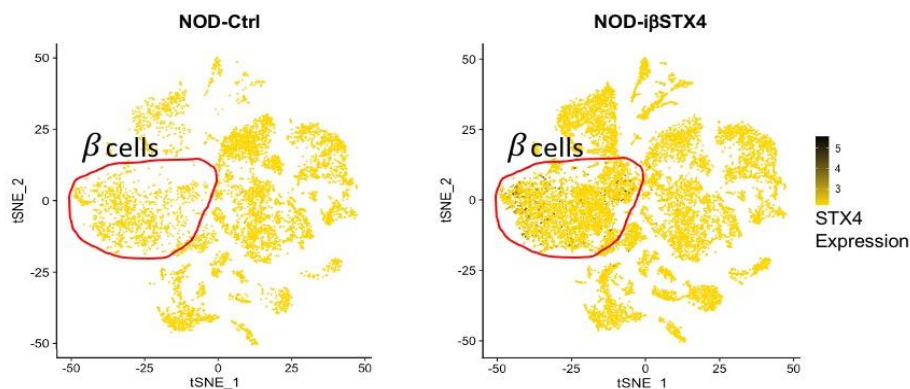
Supplemental Figure 2. Plasma insulin content and insulinitis in young 7 week old NOD and NOD-iβSTX4 mice. (A) Plasma insulin content, shown as stimulation index (10 min after glucose administration / fasted 6 hr (Glucose/Basal)), in 7 week old female Dox-induced NOD-iβSTX4 (green) vs. NOD-Ctrl mice (dTg, no Dox, yellow). Data represent the average \pm SEM of 3 mice per group. (B) Insulinitis scores were counted from 3-4 sets of littermate NOD-iβSTX4 (green) or NOD-Ctrl (yellow) mice.

Fig. S3

A. tSNE plot for NOD islets



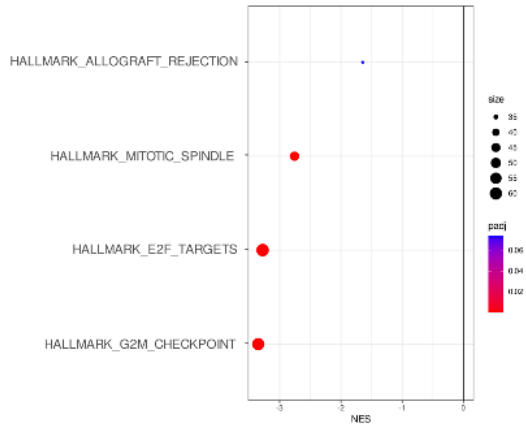
B. STX4 expression



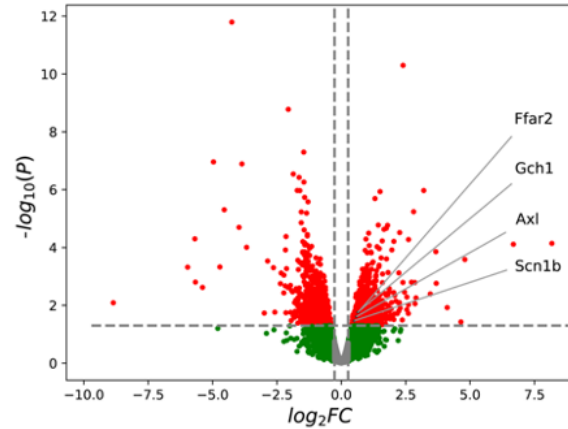
Supplemental Figure 3. A t-distributed stochastic neighbor embedding (t-SNE) plot by single-cell RNA sequencing analysis. (A) t-SNE plot was used to visualize the data in a two-dimensional subspace, which led to identification of 19 major clusters (0-18). Cluster 0: β -cells, cluster 2: α -cells, cluster 6: δ -cells, Cluster 14: endothelial cells, Cluster 15: ductal cells, cluster 1, 4, 9, 13, 16, 17: T cells and other clusters were unidentified. **(B)** Black dots indicate STX4 expression greater than 2.2-fold higher in NOD-i β STX4 mouse islet cells compared with those from NOD-Ctrl. Red outlines show defined β -cell cluster.

Fig. S4

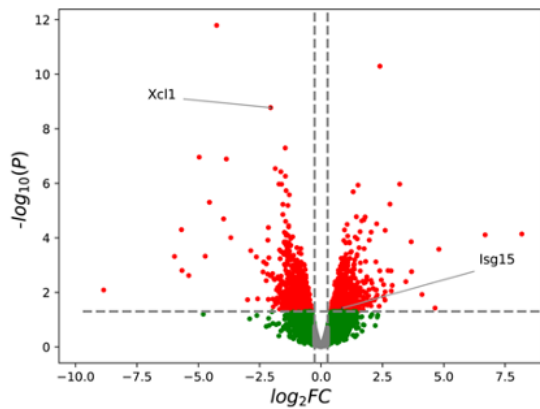
A. Hallmark GSEA in pancreatic α cells



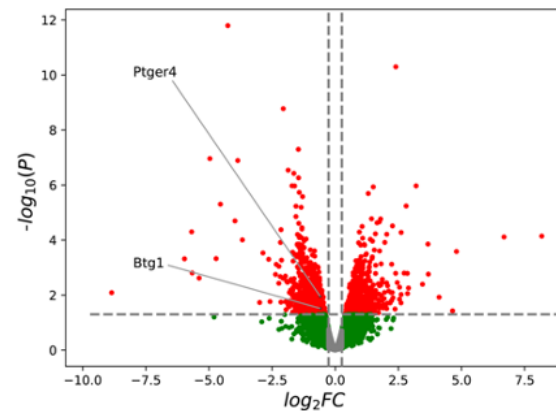
B. Inflammatory response in pancreatic α cells



C. Interferon gamma response in pancreatic α cells



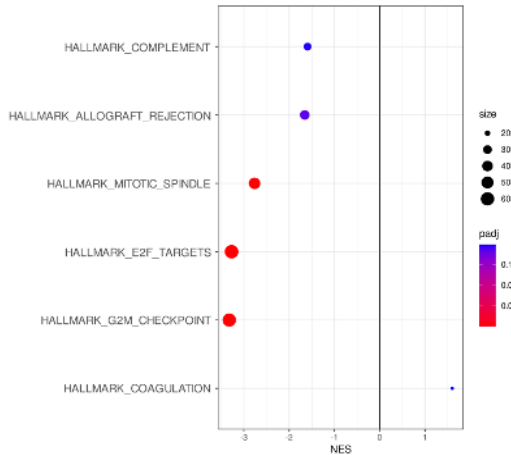
D. TNF- α signaling via NF- κ B in pancreatic α cells



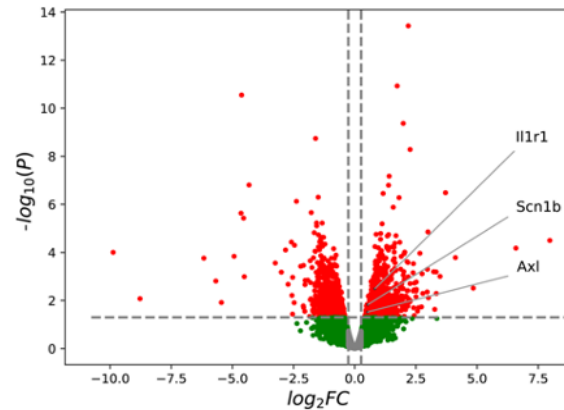
Supplemental Figure 4. Transcriptomic analysis of isolated pancreatic α -cells from NOD-Ctrl or NOD- β STX4 mice. (A) Bubble plot of Hallmark gene set enrichment analysis (GSEA) from comparisons of expression profiles between NOD- β STX4 and NOD-Ctrl in pancreatic α -cells (from 13 week old mice as described in Fig. 4). The graph shows the signaling pathways which were statistically significant. Color represents adjusted p value. The size of each bubble reflects the number of differentially expressed genes. **(B)** Volcano plot for genes in pancreatic α -cells in the “inflammatory response” gene set. Red dots indicate expression changes of $> \pm 1.2$ -fold with $p < 0.05$. green dots indicate expression changes $< \pm 1.2$ -fold with $p < 0.05$. Grey dots indicate expression changes $< \pm 1.2$ -fold with $p \geq 0.05$. FC: fold change. **(C)** Volcano plot for genes in pancreatic α -cells in the “IFN γ response” gene set. **(D)** Volcano plot for genes in pancreatic α cells in the “TNF α signaling via NF- κ B” gene set.

Fig. S5

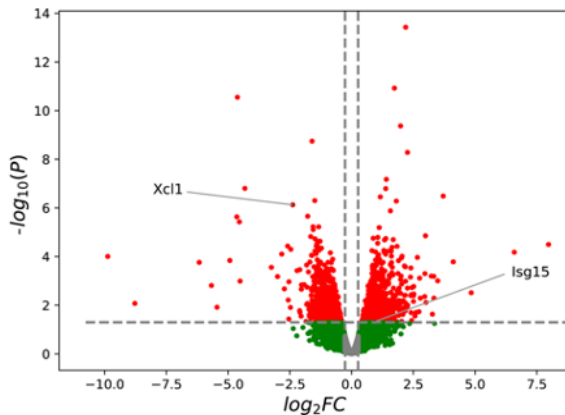
A. Hallmark GSEA in pancreatic δ cells



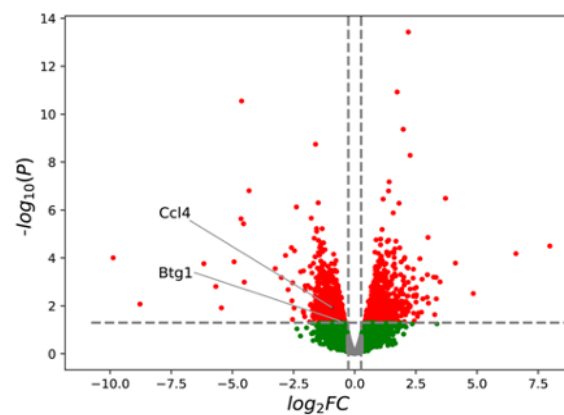
B. Inflammatory response in pancreatic δ cells



C. Interferon gamma response in pancreatic δ cells

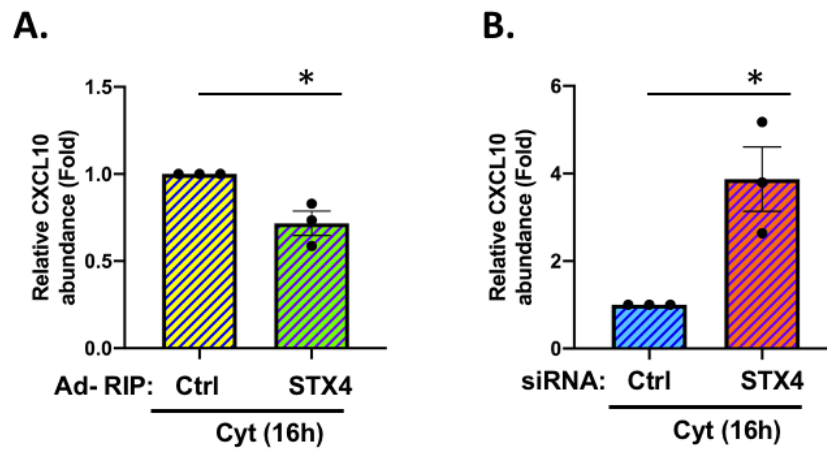


D. TNF- α signaling via NF- κ B in pancreatic δ cells



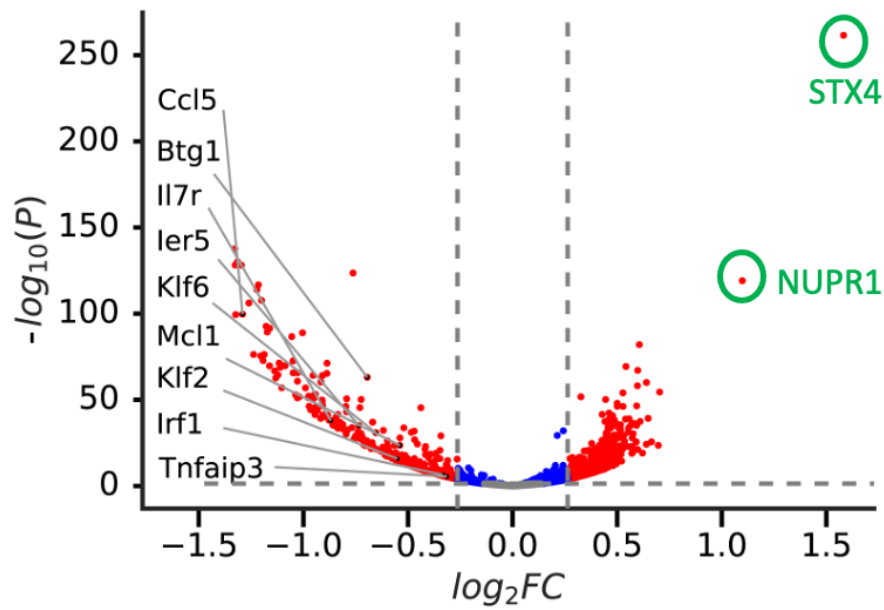
Supplemental Figure 5. Transcriptomic analysis of isolated pancreatic δ -cells from NOD-Ctrl or NOD- β STX4 mice. (A) Bubble plot of Hallmark gene set enrichment analysis (GSEA) from comparisons of expression profiles between NOD- β STX4 and NOD-Ctrl in pancreatic δ -cells (from 13 week old mice as described in Fig. 4). The graph shows the signaling pathways which were statistically significant. Color represents adjusted p value. The size of each bubble reflects the number of differentially expressed genes. **(B)** Volcano plot for genes in pancreatic δ -cells in the “inflammatory response” gene set. Red dots indicate expression changes of $> \pm 1.2$ -fold with $p < 0.05$. green dots indicate expression changes $< \pm 1.2$ -fold with $p < 0.05$. Grey dots indicate expression changes $< \pm 1.2$ -fold with $p \geq 0.05$. FC: fold change. **(C)** Volcano plot for genes in pancreatic δ -cells in the “IFN γ response” gene set. **(D)** Volcano plot for genes in pancreatic δ -cells in the “TNF α signaling via NF- κ B” gene set.

Fig. S6



Supplemental Figure 6. STX4 regulates CXCL10 mRNA levels. Effects of **(A)** STX4 overexpression or **(B)** STX4 knockdown on CXCL10 mRNA expression in cytokine-treated INS-1 832/13 β -cells. Data represent three independent experiments using distinct passages of cells; $*p < 0.05$.

Fig. S7



Supplemental Fig. 7. Expanded volcano plot for genes involved in the Hallmark “TNF α signaling via NF- κ B” pathway in β -cells, including STX4. Replot of Figure 5D, with the full-length Y-axis, to permit the visualization of the STX4 and NUPR1 genes, circled in green.