SUPPLEMENTARY MATERIAL

Long RNA sequencing.

RNA extraction was performed using Trizol. Ten million cells were used per samples. Libraries for the direct RNA sequencing were generated using the recommended protocol for the SQK-RNA001 kit (Oxford Nanopore Technologies). Libraries were loaded and sequenced on MinION R9.4 flow cells (Oxford Nanopore Technologies) for 48 hours. Base calling was performed concurrent with sequencing using Albacore (v 2.0), integrated within the MinION software (MinKNOW, v1.10.23). Only "pass" reads were used for subsequent analyses.SQK-RNA002 en MinION R9.4.1 flow cells were used for a run time of 72 uur. Sequences were analysed using GridION software version MinKNOW core 3.6.5 Bream 4.3.16 GUI 3.6.16 Guppy 3.2.10 Script Configuration 1.0.9. The nanopore PASSED fastq files were processed using an inhouse BioWDL pipeline TALON-WDL (https://github.com/biowdl/TALON-WDL). This pipeline uses minimap2 version 2.17 (https://github.com/lh3/minimap2) for the alignment with the reference genome GRCh38. Minimap2 settings for noisy nanopore direct RNA-seq were used as recommended by the developer. The alignments are further polished using TranscriptClean version 2.0.2 (https://github.com/mortazavilab/TranscriptClean). Splice junctions were corrected according to known splice junctions that were extracted from the GENCODE gene annotation version 33. Known variants were preserved based on https://ftp.ncbi.nih.gov/snp/organisms/human_9606/VCF/00-common_all.vcf.gz. The final identification of known and novel transcripts was done with the platform agnostic tool TALON version 4.4.2 (https://github.com/mortazavilab/TALON). In order to remove noisy and potential false positive transcripts, the following filtering steps were executed on the TALON output abundance file: transcripts showing mean reads equal or higher than 10 within triplicates, per condition, were included in the analysis and TALON transcripts deriving from

incomplete splice matches reads or shorter than 30 bp were excluded from the analysis. A paired negative binomial generalized log-linear model was used to identify DEGs in the Nanopore data.

Alignments and P-site detection.

All Ilumina reads were quality controlled and adapter sequences (CTGTAGGCACCATCAAT) were clipped using cutadapt (version 2.8) (https://cutadapt.readthedocs.io/en/stable/index.html). The cleaned reads were aligned to the transcriptome reference with of ENSEMBL build GRCh38.99 for the controls (N=3) and cytokine samples (N=3) using STAR version 2.7.3a (https://pubmed.ncbi.nlm.nih.gov/23104886/) The following STAR settings were used: "--outReadsUnmapped --twopassMode Basic --quantMode TranscriptomeSAM --chimJunctionOverhangMin 15 --chimSegmentMin 15". The R-package riboWaltz (v1.1.0) was used to calculate the P-site offsets to identify the position of the ribosome P-site. A length filter of 26-30 nt was applied. A FASTA file (build GRCh38.99) was downloaded from ENSEMBL to identify the sequences of the P-site, E-site and A-site. To identify P-sites that were enriched over adjacent background a dynamic sliding window was used. The size of the window for a P-site *i* was defined as: $Li = 5 \cdot \log2(\Sigma\psi)$. Where L is the size in nucleotides, Σ the number of counts at P-site ψ. The window sizes were limited to a minimum of 55 to a maximum of 50 nucleotides and a minimal of 4 P-sites in the window. Next, a paired negative binomial generalized log-linear model as implemented in the R-package edgeR (v3.28.1) was used to test the P-site expression against the median of the background while taking library size into account. The enrichment over background was performed for the control and cytokine group separately. The same enrichment and differential expression procedure was performed for the alignment based on the Nanopore data. To find differentially expressed P-sites between

controls and cytokine exposed samples, a paired negative binomial generalized log-linear model was fitted with library size taken into account. This analysis was performed for the ENSEMBL based alignment and the Nanopore based alignment.

Alignment of nanopore reads to primary human beta cell RNAseq

In order to evaluate the novel transcripts detected by the TALON-WDL pipeline using Nanopore data, we performed an in-silico validation by examining support reads to these novel transcripts using a different Illumina based bulk RNAseq dataset. This RNAseq dataset contains 12 non-diabetic and 4 type 1 diabetic beta cell samples (GEO GSE121863). Illumina short reads were aligned to the GRCh38 reference genome using STAR version 2.7.3a with the two-pass mode (--twopassMode Basic). By combining all splicing junctions indicating intron events in these 12 healthy beta cell samples and 4 type 1 diabetic samples detected by STAR, we identified in total 334686 introns and 264282 introns respectively supported by at least 1 high confidence Illumina splicing read. All known introns from the GENCODE version 33 GTF file were extracted and compared to the detected introns by the TALON-WDL pipeline (listed in the "nanopore_DGE" and "nanopore_DGE_T1D_genes" of Data file S3 from the Nanopore reads using the CGAT gtf2gtf program (https://cgat.readthedocs.io/en/latest/cgat.html).

Islet donors

Pancreatic islets were obtained from human organ donor pancreata. Human islets were isolated from organ donors. Islets were only studied if they could not be used for clinical purposes and if research consent had been obtained. According to the national law ethics approval is not required for research on donor tissues that cannot be used for clinical transplantation. The isolations were performed in the GMP-facility of LUMC according to the previously described

protocol (1). For experimental use, human islets were maintained in ultra-low attachment plates (Corning, NY 14831) in low glucose DMEM supplemented with 10% FBS, 100 units/ml Penicillin and 100 µg/ml streptomycin. Dispersed islet cells were treated with 1000 U/ml IFNγ, 2 ng/ml IL1βfor 24 hours. All methods were carried out in accordance with relevant guidelines and regulations.

Cells and reagents.

JY cells expressing HLA-A2 were maintained in IMDM supplemented with 10% FBS, 100 units/ml Penicillin and 100 µg/ml streptomycin. For pulsing, 2 µg/ml peptide were added on 10×10^6 cells / ml in AIM-V medium (Life Technologies) for 2 h at 37 °C. JY cells expressing HLA-A2 were maintained in IMDM supplemented with 10% FBS, 100 units/ml Penicillin and 100 µg/ml streptomycin. For pulsing, 2 µg/ml peptide were added on 10×10^6 cells / ml in AIM-V medium (Life Technologies) for 2 h at 37 °C. All the peptides used in this study are listed in the table below:

Peptide	Sequence	Category
PPI_{15-24}	ALWGPDPAAA	Canonical
$INSB_{10-18}$	HLVEALYLV	Canonical
$INS-DRIP_{1-9}$	MLYQHLLPL	Alternative (+2 frame shift)

Lentiviruses Production and Transduction

The vectors were produced as described previously(2). Viral supernatants (MOI=2) were added to fresh medium supplemented with 8 mg/mL Polybrene (Sigma-Aldrich), and the cells were incubated overnight. The next day, the medium was replaced with fresh medium. Transduction efficiency was analyzed 3–6 days after transduction. The lentiviral vector containing HLA-

A02:01 under elongation factor 1α (EF1α) promotor has been obtained from R. J. Lebbink (Medical Microbiology, University Medical Center Utrecht, Utrecht, the Netherlands)(3).

T-Cell Activation Assays

Target cells were harvested and cocultured in suspension with CTLs specific for INS-DRiP$_{1-9}$ (MLYQHLLPL) at a ratio 1E:1T, in the presence of mouse anti-human CD107a- FITC (11-1079-42; Thermo Fisher Scientific). Cocultures were incubated at 37°C for 4 h in IMDM supplemented with 10% human serum and 40U/ml IL-2 (Novartis). Cells were washed with flow cytometry buffer (0.5% human albumin, 0.01% Na azide, PBS), stained for CD8-APC (BD Biosciences), and analyzed by flow cytometry. The degranulation of T cells was calculated as a ratio of percentage of CD107a$^+$ cells/percentage of total CD8$^+$ cells.

The supernatant was used for detection on MIP-1β production by the T cells, using the MIP-1β ELISA kit (# 88-7034-22; Thermo Fisher Scientific), according to the manufacturer's protocol.

DNA constructs and fusion protein analysis

(Alt-CHGA)-GFP construct, containing the 5'-UTR region of CHGA, has been designed with Snapgene and purchased at genescript (Invitrogen). The fragment was cloned into pcDNA3.1 vector to allow expression in eukaryotic cells. The vector has been transfected into HEK 293 cells and EndoC-BH1 cells using Lipofectamine 2000 (Invitrogen, 11668-019). After 24h, cells were treated with IFNg/IL1b. Forty-eight hours post transfection, the cells were analysed by western blot.

Western Blot Analyses

Cells were lysed in RIPA buffer supplemented with protease inhibitor cocktail (Roche). Protein quantification was performed with the BCA protein assay kit (Thermo Fisher Scientific). To verify the expression of proteins induced by cytokines (CHGA, PSMB8, PSMB9, PSMB10, STAT1 and STAT2) 15 µg proteins extracts were loaded on 10% acrylamide/bis acrylamide SDS page gel. For the detection of the upstream ORF of CHGA-GFP 50 µg proteins extracts were loaded on 15% acrylamide/bis acrylamide SDS page gel. After electrophoresis, protein transfer was performed on a nitrocellulose membrane (GE Healthcare). Membranes were stained with primary antibodies overnight at 4°C and secondary HRP conjugated antibodies (Santa Cruz Biotechnology) for 1h RT. Western ECL substrate was used for imaging (#1705062, BioRad). All antibodies used in this study are listed in the table below.

Protein name	**Antibody**	**dilution**	**Manufacturer**
CHGA	#HPA017369	1:500	Atlas antibodies
STAT1	#14995	1:1000	Cell Signaling Technology
STAT2	#72604	1:1000	Cell Signaling Technology
LMP2 (*PSMB9* gene)	#BML-PW8345	1:1000	Enzo Life Sciences
LMP7 (*PSMB8* gene)	#BML-PW8355	1:1000	Enzo Life Sciences
MECL-1 (*PSMB10* gene)	#BML-PW8350	1:1000	Enzo Life Sciences
GFP	#A11122	1:1000	Invitrogen
Actin	#0869100	1:5000	MP Biomedicals

RT-PCR

Total RNA was extracted from ENDOC-βH1 using TRIzol reagent and from islets using RNeasy micro kit (QIAGEN) following the manufacturer's instructions. Isolated RNA was quantified using a Nanodrop 1000 spectrophotometer. Approximately 1ug of RNA was treated with DNase I (Thermo Fisher Scientific). 500 ng of DNase treated RNA was reverse transcribed using the Superscript RT II kit (Invitrogen), while 500ng was used as a negative control of reverse transcription. Oligo (dT) primers were used in the reaction. Expression of the transcript of interest was detected using the following primers: INS-IGF2 (TALONT000499153) Forward: GAGAACTACTGCAACTAGACGCAG, INS-IGF2 (TALONT000499153) Reverse: CTACTGAACAAGAAGTCACTTCTCAG; PSMB9 (TALONT000709680) Forward: CACACCGGGGTAATGGGTC, PSMB9 (TALONT000709680) Reverse: GGGCACCATTTCCTCATCTC; HLA-C (TALONT000700063) Forward: GGGGTCACATCTCTTATCAGA, HLA-C (TALONT000700063) Reverse: CCTACACATCATAGCGGTG; GAPDH Forward: CCTGTTCGACAGTCAGCCG, GAPDH Reverse: CGACCAAATCCGTTGACTCC.

PCR amplification was performed in a volume of 10 µl, containing 0.5 µM of each primer, 1X Flexi buffer Polymerase (Promega) , 200 µM dNTPs Polymerase (Promega), and 0,5 U of Gotaq Flexi DNA Polymerase (Promega). PCR was performed in a T100 Thermal Cycler (Bio-Rad). Initial denaturation was performed at 95 °C for 2 min, followed by 5 cycles consisting of denaturation at 94 °C for 30 s, annealing at 65 °C for 30 s (decreased with -1°C/ cycle) and extension at 72 °C for 1 min. The subsequent 30 cycles consisted of denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s and extension at 72 °C for 1 min Finally, a 2 min extension step was performed at 72 °C. For visualisation, the amplification products were run on 1.5 % (w/v) agarose MP gel (Roche Diagnostics GmbH, Mannheim, Germany). The amplicon size was estimated using the Generuler 1 kb DNA Plus Ladder (ThermoFisher Scientific). The amplicons were excised and isolated from agarose gel using the Nucleospin Gel & PCR Clean-

up kit (Bioke) following manufacturers protocol. The isolated fragments were cloned using the pGEM-T Easy Vector System (Promega) following manufacturers protocol, and subsequently transformed into competent cells. IPTG and β-galactosidase were added to the plate in order to enable blue/ white colony screening. White colonies were selected, Plasmid DNA was isolated using the PureYield Plasmid Miniprep Kit (Promega) using manufacturers protocol. The isolated plasmids were analysed by sequencing using the M13 sequencing primers.

REFERENCES

1. Ricordi C, Lacy PE, Finke EH, Olack BJ, Scharp DW: Automated method for isolation of human pancreatic islets. Diabetes 1988;37:413-420

2. Carlotti F, Bazuine M, Kekarainen T, Seppen J, Pognonec P, Maassen JA, Hoeben RC: Lentiviral vectors efficiently transduce quiescent mature 3T3-L1 adipocytes. Mol Ther 2004;9:209-217

3. van der Torren CR, Zaldumbide A, Duinkerken G, Brand-Schaaf SH, Peakman M, Stange G, Martinson L, Kroon E, Brandon EP, Pipeleers D, Roep BO: Immunogenicity of human embryonic stem cell-derived beta cells. Diabetologia 2017;60:126-133