# Supplemental Information

# Identification of a SIRT1 Mutation in a Family with Type 1 Diabetes

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## Supplemental Information Inventory

Supplemental Data

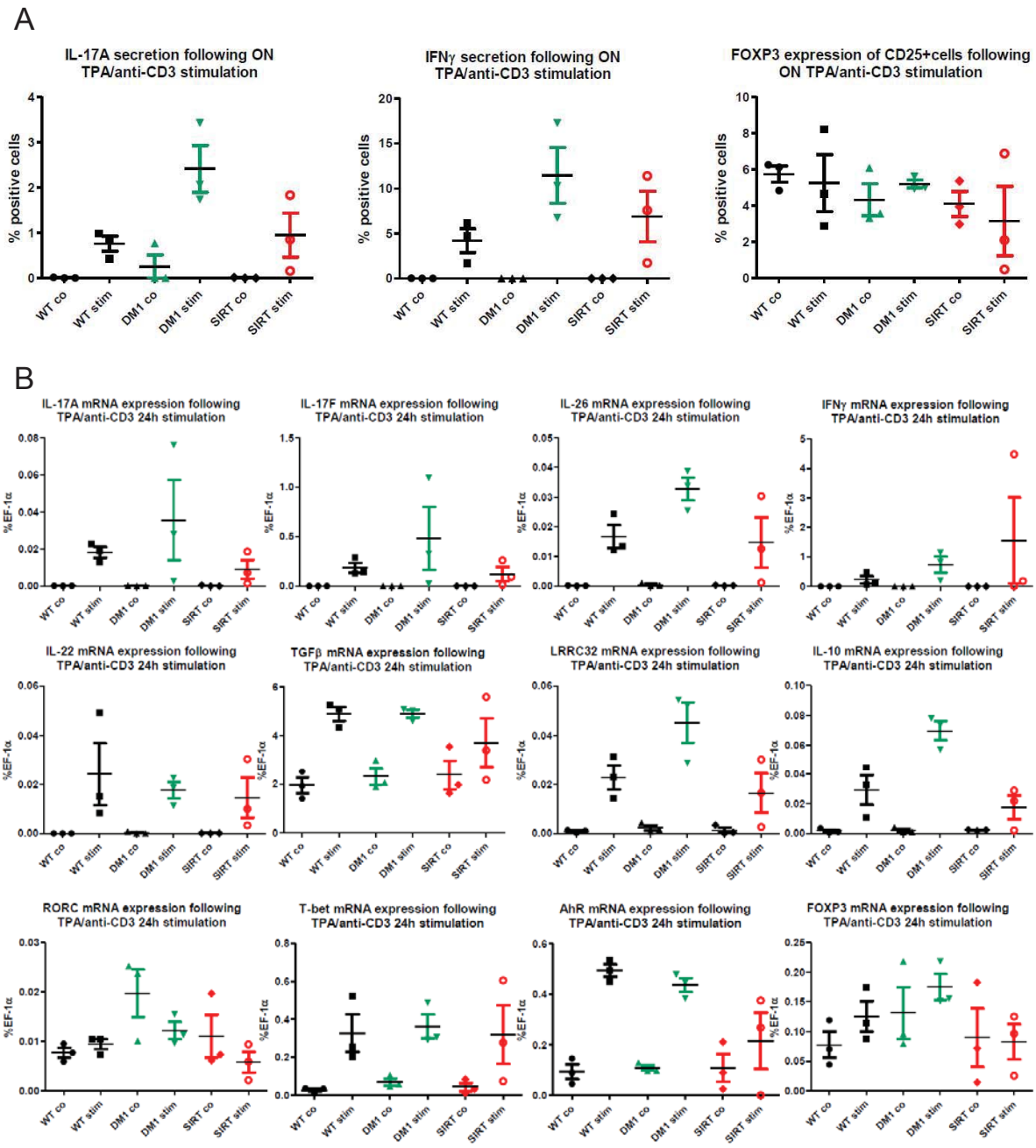
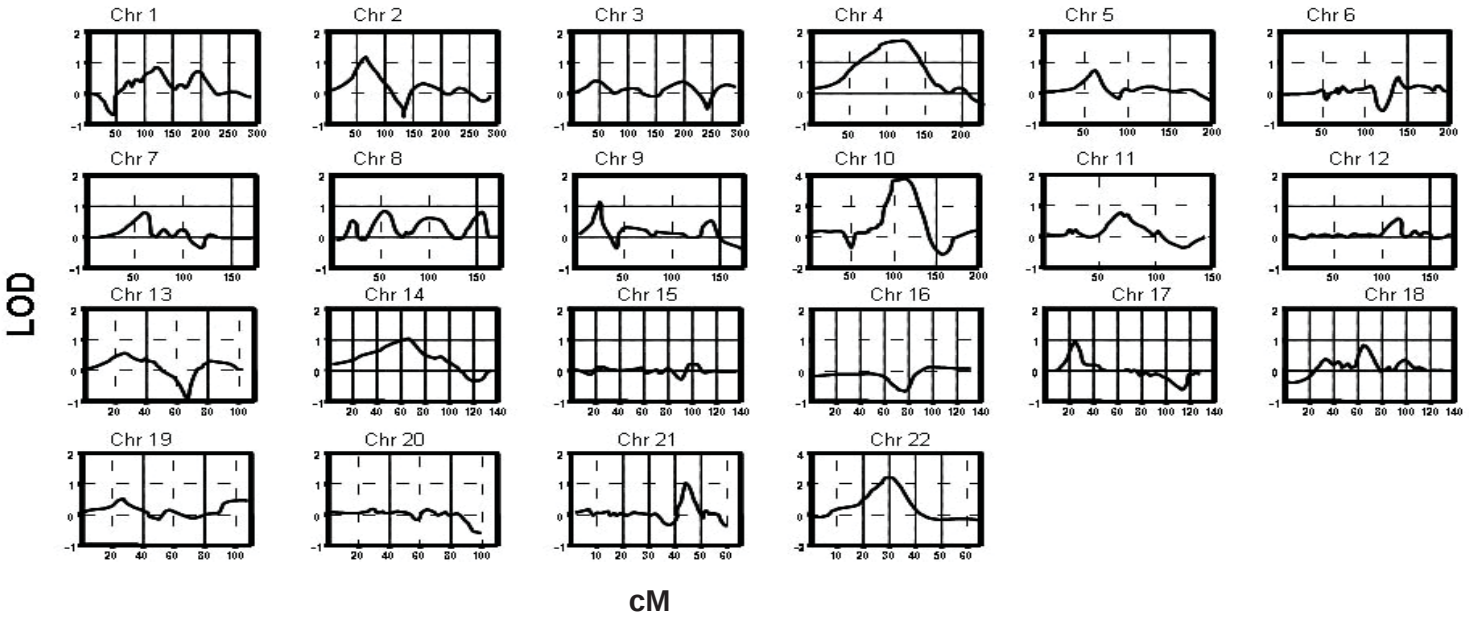



**Figure S1. Functional analysis of human peripheral blood mononuclear cells.**

Cells were obtained from healthy control subjects (WT) and from patients with type 1 diabetes carrying wild type SIRT1 (DM1) or the L107P mutation (SIRT). (A), protein analysis at single cell level determined by FACS and (B), mRNA transcription analysis assessed by quantitative RT-PCR, following anti-CD3/TPA stimulation of purified $CD4^+$ Th cells. n=3 for each of the three groups.

A




B

## EXOME SEQUENCING

**p845: whole exome sequencing**

**Two selected position with SNPs that are present only in 2 affected patients**

| | | | | | 7_IV-4_WT | | | | | 6_IV-5_WT | | | | | 1_IV-2_mut | | | | | 8_III-1_mut | | | | | 5_III-4_mut | | | | | 2_IV-8_WT | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BitFlag | #samples | Chrs | SNP pos | reference base | genotype | variants | coverage | reference allele counts | novel allele counts | genotype | variants | coverage | reference allele counts | novel allele counts | genotype | variants | coverage | reference allele counts | novel allele counts | genotype | variants | coverage | reference allele counts | novel allele counts | genotype | variants | coverage | reference allele counts | novel allele counts | genotype | variants | coverage | reference allele counts | novel allele counts | Name | Accession | start | stop | chrs | strand |
| 33 | 2 | chr10 | 72300885 | C | - | - | - | - | - | - | - | - | - | - | Y | C/T | 17 | 5 | 11 | Y | C/T | 19 | 11 | 6 | - | - | - | - | - | - | - | - | - | - | KIAA1274 | NM_014431 | 72300858 | 72300998 | chr10 | + |
| 33 | 2 | chr10 | 73521578 | G | - | - | - | - | - | - | - | - | - | - | A | A | 8 | 0 | 7 | R | G/A | 23 | 18 | 5 | - | - | - | - | - | - | - | - | - | - | C10orf54 | NM_022153 | 73521355 | 73521783 | chr10 | - |

**DIRECT SEQUENCING**

KIAA1274= Paladin (http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2731165/pdf/pone.0006871.pdf) Exon 16

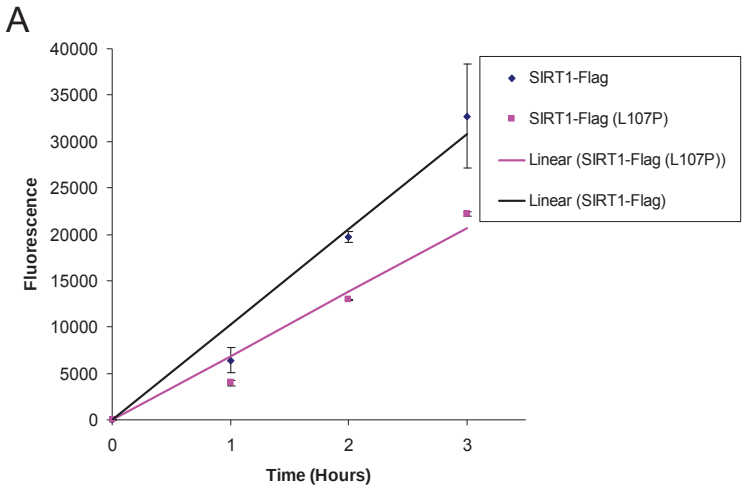
**Affected** IV-2 heterozygous C/T * *(known SNP rs117803924 c.2217c>t non synonymous change p.R646W)
III-1 heterozygous C/T
III-4 homozygous C
IV-9 homozygous C
IV-1 homozygous C

**Non affected**: III-3 heterozygous C/T
IV-4 homozygous C
IV-5 homozygous C
IV-8 homozygous C

**Controls** **unaffected** (n=50) 20/50 C/T 30/50 C **Ashkenazy Jews** (n=20) 12/20 C/T 8/20 C **T1DM white** (N=50) 23 C/T 27 C

**Figure S2. Genome-wide linkage analysis and analysis of the KIAA1274 variant.**

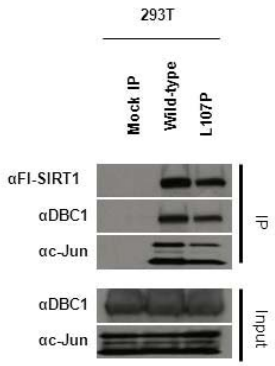
(A) The LOD score in genome-wide linkage analysis using 811 microsatellite markers (ABI prism linkage mapping set HD5 v2.5) covering 22 chromosomes. Each box represents a chromosome. The *x* axis gives the genetic distance in cM, and the *y* axis gives the LOD scores. Significance in whole genome microsatellite mapping LOD >2.2. Chromosome 10 (LOD 4.0 between D10S210 and D10S537) Chromosome 22 (LOD 2.2). (B) Roman numerals refer to the individuals identified in Figure 1.




B

Top SIRT1 Interactors
(LC-MS/MS)

| Protein Name | # Peptides |
|---|---|
| SIRT1 | 75 |
| DBC1 | 34 |
| TIF1b | 26 |
| DDX56 | 13 |
| Jun(c,d) | 9 |



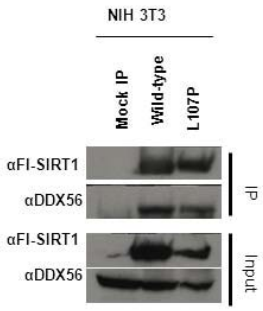




**Figure S3. Measurement of SIRT1-L107P deacetylase activity and detection of SIRT1 and SIRT1-L107P protein-protein interactions**

(A) Equal amounts of Flag-tagged wild-type SIRT1 (diamonds) or Flag-tagged SIRT1-L107P (squares) were produced and purified from 293T cells. Enzymatic activity was assessed using the BIOMOL Fluor de Lys kit at several time intervals (0, 1, 2, 3 hours). (n=3) All data are presented as mean ± SEM. (B) Identification of the top SIRT1 interacting partners via LC-MS/MS. (C) Comparison of SIRT1- Wt and L107P interactions with DBC1 and c-Jun in 293T cells. (D) Comparison of SIRT1- Wt and L107P interactions with DDX56 in 3T3 cells

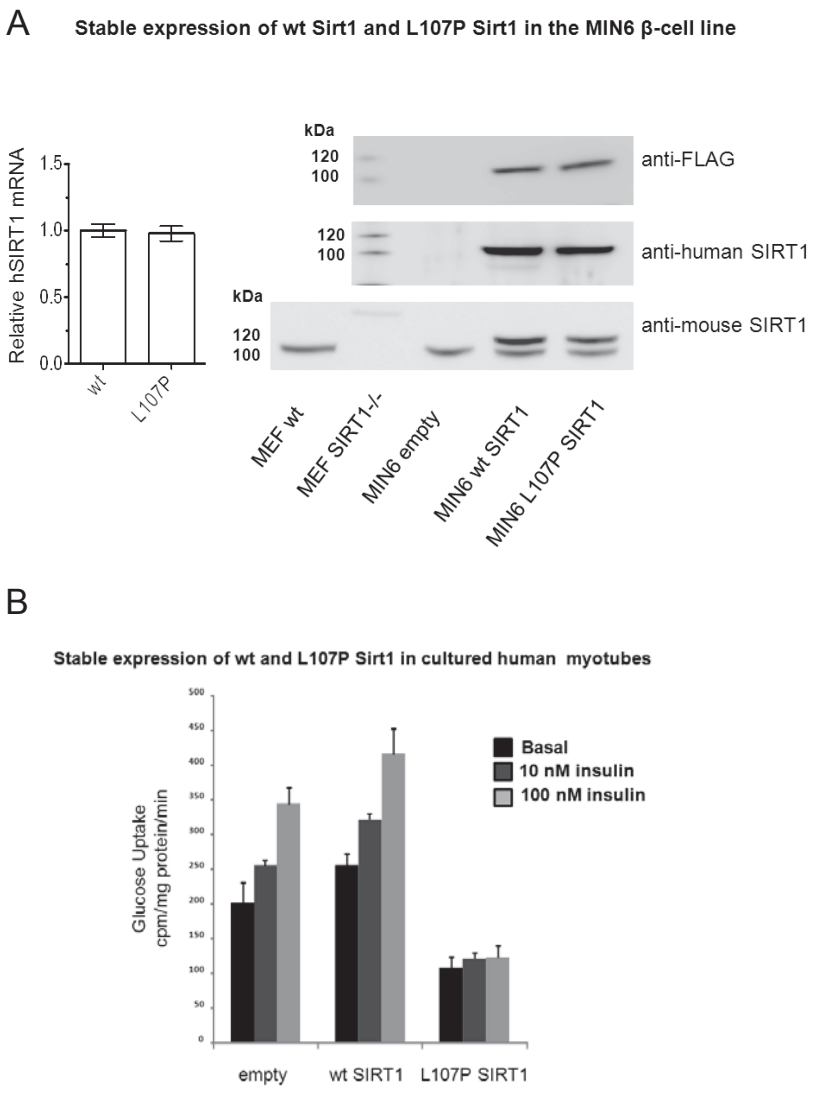



**Figure S4. Western blots of MIN6 cells overexpressing Flag tagged wt SIRT1 or L107P SIRT1 and Impaired insulin sensitivity in human skeletal myotubes overexpressing L107P SIRT1**.

(A) Protein extracts of MIN6 empty, MIN6 wt SIRT1, MIN6 L107P SIRT1, wt Mouse embryo fibroblasts (MEF) and SIRT1 deficient MEFs were analysed by Western blotting using an anti-mouse SIRT1, an anti-human SIRT1, and an anti-FLAG antibody. Equal protein loading of the blots was verified by Ponceau staining. Note that the anti-mouse SIRT1 antibody crossreacts with the FLAG-tagged human SIRT1. Taq Man real time PCR data with primers specific for human SIRT1 further confirmed that the SIRT expression level was comparable between wt and L107P overexpressing cells. (B) Measurement of insulin stimulated glucose uptake of cultured human skeletal myotubes obtained from biopsies of the vastus lateralis muscle of non-transduced healthy individuals, and those transduced with wild type (wt) and L107P SIRT1 (n=3). All data are presented as mean ± SEM.

**Table S1**: Pattern of hybridization of DBQ1 sequence-specific probes. The "+" sign indicates hybridization of the probe to the sequence

| **DQB1 Allele** | **AD01 27** | **AD01 28** | **AD01 29** | **AD01 30** | **AD01 31** | **AD01 32** | **AD01 50** | **AD01 51** | **AD01 52** | **AD01 53** | **AD01 54** | **AD01 55** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 02 | | | + | | | + | | | | | | |
| 0301,0309 | | | + | | + | | | | + | | | |
| 0302,0307 | | | + | + | | | | | | | | |
| 0303, 0306, 0310 | | | + | | | | | | + | | | |
| 0304 | | | + | + | + | | | | | | | |
| 03051 | | | + | + | | | | + | | + | | |
| 03052 | | | + | + | | | | + | | | | |
| 04 | | | + | | | | | + | | + | | |
| 05011 | | | + | | | | | + | | | + | |
| 05012, 0503 | | | + | | | | | + | | | | |
| 0502, 0504 | | | + | | | | | + | | | | |
| 0601 | | | + | | | | + | | | | | |
| 0602, 0611, 0613, 0615, 06042 | + | | + | | | | | | | | | |
| 0603, 0607, 0608, 0614, | + | + | + | | | | | | | | | |
| 0604, 0617 | | + | + | | | | | | | | | |
| 0610 | + | | + | | | | | | | | | + |
| **Subject** | | | | | | | | | | | | |
| IV-1 | | | ✓ | ✓ | | ✓ | | ✓ | | ✓ | | |
| III-1 | | | ✓ | | ✓ | | | | ✓ | ✓ | | |
| III-3 | | | ✓ | | ✓ | | | | ✓ | | | ✓ |
| III-4 | | | ✓ | ✓ | ✓ | | | | ✓ | | | |
| IV-9 | | | ✓ | ✓ | ✓ | | | | ✓ | ✓ | | |
| IV-5 | | | ✓ | ✓ | | | | | ✓ | ✓ | | |
| III-9 | ✓ | | ✓ | | | | ✓ | | | | | ✓ |
| CONTROL | | ✓ | ✓ | | ✓ | | | | ✓ | | | |

## SUPPLEMENTAL EXPERIMENTAL PROCEDURES

### Mutation analysis.

Genomic DNA was extracted from peripheral blood leukocytes. DNA from 100 unrelated individuals (80 Caucasian, 8 of whom from Turkey; 10 Asian; 10 Blacks, 2 of whom from Central America and 2 Hispanic) was used as control for polymorphisms. *HLA-DQB1* analysis was performed using the DELFIA Hybridization Assay (Wallac Oy, Turku, Finland) and Microsatellite analysis was performed using the ABI Prism Linkage mapping set version 2.5 (811 markers, Applied Biosystems). Haplotypes were reconstructed by using the GENEHUNTER package (Version 1.2). The disease gene frequency in the general population was set at 0.0001. Two-point and multipoint linkage analyses were performed by GENEHUNTER by using the affected-only allele-sharing method. SLink simulations were done by the FASTLINK package. For direct sequencing, the coding regions of *SIRT1* (NC_000010.9 region 69314433..69348149), *TCF7L2* (NC_000010.9), *IRS2* (NC_000013.9) and *SOCS2* (NC_000012.10) were amplified and the PCR products were sequenced using the Big Dye Terminator Cycle Sequencing Kit and analyzed on an ABI Prism 310 Genetic Analyzer (Applied Biosystems). Sanger sequencing for the validation of the human exome resequencing of SNP rs.117803924 (c.[2217C>T], p.Arg646Trp) in exon 16 of KIAA1274 (Paladin NM_014431 region 72300858..72300998) gene present in at least two family members, was performed in affected (n=5) and non-affected (n=4) family members, normal individuals (n=50), ethnic matched controls (Ashkenazi Jews n=20) and Type 1 diabetic patients (white n=50). Nucleotide and amino acid exchanges are named according to http://www.hgvs.org/mutnomen/.

To confirm that the SIRT1 mutation is causative we also performed whole-exome sequencing. The all exome enriched library was produced using the SOLiD Fragment Library Construction kit (Applied Biosystems p/n 4443471) and the SureSelect Human exome kit (Agilent p/n G3362B). The exome design targets all human exon regions, totaling approximately 38 Mb. 3 µg of genomic DNA was fragmented to a size range of 100–150 bp with the use of a Covaris Instrument S2 (Covaris) and analyzed on an Agilent Bioanalyzer 2100 DNA Chip 7500. Fragments were end-repaired using polishing enzymes and ligated with truncated adaptors in presence of T4 DNA Ligase. The ligated DNA was size selected to approximately 200bp on an E-Gel device (Invitrogen p/n G6465). The ligated DNA was nick

translated and amplified. After quality control, the library was hybridized to biotinylated cRNA oligonucleotide baits, and subsequently purified by streptavidin-bound magnetic beads (Invitrogen p/n 656-01). The purified sample was amplified by ligation-mediated PCR using primers complementary to the sequence of the SOLiD 4 full length adaptors. The enriched library was used for e-PCR based on a concentration of 0.5pM. A single oct of the SOLiD 4 sequencing slide (Applied Biosystems) was used for each sample. Average coverage 18.

For the sequence analysis, the colour space sequence reads (50bp) were mapped to the human genome assembly hg19 (ftp://hgdownload.cse.ucsc.edu/goldenPath/hg19/bigZips/chromFa.tar.gz) using the software Bioscope 1.2.1 from Applied Biosystems (Life Technologies, Carlsbad, CA, USA). After mapping, the single nucleotide polymorphisms (SNPs) were identified by using the DiBayes algorithm (Life Technologies, Carlsbad, CA, USA) with mediumstringency settings. The obtained SNPs were classified into intronic, intergenic, coding (synonymous and non-synonymous amino acid substitutions) and splice-site variants with a custom software pipeline. Furthermore, all SNPs were compared to dbSNP build 130, OMIM and clinically relevant mutations.

For a High-Throughput sequencing of genomic DNAs, two thousands Danish patients with type 1 diabetes diagnosed before the age of 16 years including affected sib-pair and parent-offspring families were collected and used to amplify SIRT1 exon 1. Amplification and sequencing were performed as a fee-for-service by Microsynth (Balgach, CH).

**Functional analysis on human peripheral blood mononuclear cells**

Peripheral blood mononuclear cells were isolated from age matched healthy control, type 1 diabetes and SIRT1-L107P mutated donors using standard Ficoll gradient separation procedures. Aliquots of $4x10^4$ cells were seeded in triplicate wells and stimulated for 48h with 5µg/mL GAD65 515-528 (Abcam #ab457240); 5µg/mL GAD65 protein (Abnova #H00002572-P02) or 10µg/mL insulin (Sigma #I9278). Enzyme Linked Immuno Spot Assay was performed using a kit from MABTECH (#FS-0103-2). Antigen-specific IFN-$\gamma$ producing cells were identified with the help of an AID i-spot spectrum reader.

Alternatively, aliquots of $1x10^6$ $CD4^+$ Th cells from the different donors were stimulated (or not, for negative control) with 2ng/mL 12-O-tetradecanoylphorbol-13-

acetate (TPA; Sigma P1585-1MG) and 2µg/mL anti-CD3 mAb (Clone SPV-T3, Zymed Laboratories) in 48 well plate for 24hours (for RNA isolation and gene expression) or overnight in the presence of Monensin (Golgi block from eBioscience EBIO00-4505-51), which was added 3h after the stimulation (for intracytopasmic cytokine staining). RNA was isolated using a RNeasy kit (QIAGEN) and gene expression was analyzed by one step RT-QPCR (ABI Prism 7500, Applied Biosystems). The following primer probe sets were purchased by Applied Biosystemswere run in duplicates, EF1α was used as endogenous control to calculate relative gene expression. Intra Cytoplasmic staining was performed using FACS buffer (PBS containing 2% fetal bovine serum and 2mM EDTA), Fix/Perm and Permeabilization buffers from eBioscience (00-5223, 00-8333 respectively). The following antibodies were used: anti-FOXP3 AF488 (eBioscience 534776), anti-IL-17A PE (eBioscience 12-7179-42), anti-CD4 PerCP (Biolegend 300527), anti-CD25 APC (Invitrogen MHCD2505) and anti-IFNγ PECy7 (BD 557643). Samples were analyzed in a FACS CANTO II (BD Biosciences).

**RNA extraction and real time PCR**

RNA was extracted using the NucleoSpin kit (Macherey-Nagel GmBH, Oensingen, Switzerland) and reverse transcribed using the Superscipt$^{TM}$ II Rnase H$^{-}$ reverse transcriptase kit (Invitrogen Ltd., Basel, Switzerland) and random hexamer primers (Microsynth, Balgach, Switzerland). Quantitative PCR was done with TaqMan gene expression assays and the real-time PCR system 7500 of Applied Biosystems.