## SUPPLEMENTARY METHODS

### Cell culture and cytokine exposure

Pancreatic islets were isolated from adult male Wistar rats (Charles River Laboratories Belgium, Brussels, Belgium), housed and used according to the guidelines of the Belgian Regulations for Animal Care. The Ethical Committee for Animal Experiments of the Université Libre de Bruxelles approved all the utilized experimental protocols. Islets were isolated by collagenase digestion followed by hand picking under a stereomicroscope; the islets were then dispersed and β-cells purified by autofluorescence-activated cell sorting (FACS, FACStar, Becton-Dickinson and Co., Sunnyvale, CA, USA) (1;2). The preparations used in this study contained 91 ± 1% β-cells (n=38) and were pre-cultured for 36-48h in HAM's F-10 medium + 5% heat-inactivated fetal bovine serum (FBS) (2;3). Unless specified, all experimental treatments were performed in HAM's F-10 medium without serum

The following cytokines were used: recombinant human IL-1β (50U/ml, a kind gift from Dr. C.W. Reinolds, National Cancer Institute, Bethesda, MD-USA); recombinant murine TNF-α (1000U/ml, Innogenetics, Gent-Belgium) and recombinant rat IFN-γ (500U/ml, R&D systems, Oxon-UK). Cytokine concentrations were selected based on our previous dose response experiments (4;5). The time course used for cytokine treatment are indicated in each figure. In experiments in arginine-free medium, β-cells were pre-cultured in this medium for 12h and then exposed to cytokines for 24h in the presence or absence of citrulline (6).

### Viability assay

The percentage of viable, necrotic and apoptotic cells was determined in at least 600 cells in each experimental condition, by inverted fluorescence microscopy after addition of the DNA dyes Hoechst 342 (10 µg/ml) and propidium iodide (10 µg/ml) (7;8). Viability was evaluated by at least two observers, one of whom was unaware of sample identity. The agreement between findings obtained by the two observers was at least 90%.

### Western blot

Total protein extracts were obtained from β-cells exposed to cytokines and transfected or not with siRNAs, as indicated in the Figures. Lisates were subjected to a 10% SDS-PAGE and transferred to a nitrocellulose membrane. Immunoblot analysis was performed with anti-IRF-7 (Invitrogen, Camarillo, CA), anti-ATF3 (Santa Cruz Biotechnology, Santa Cruz, CA), anti-P-AKT and anti-tubulin (Cell Signaling, Beverly, MA) antibodies and then with a secondary anti-rabbit horseradish peroxidase-labeled anti-IgG (Santa Cruz Biotechnology or Cell Signaling). For quantification, the specific bands recognized by the antibodies were quantified by Scion Image (Scion Corporation, Frederick, MD). The intensity values for the proteins analyzed were corrected by the values of the housekeeping protein tubulin. Results of each experiment were normalized by the peak value considered as 1, and expressed as mean ± SEM for 3-4 independent experiments.

### NO and CCL5 measurement

Culture medium was collected for nitrite determination (nitrite is a stable product of NO oxidation) by the Griess method (9) or for assessment of CCL5 production by enzyme-linked immunosorbent assay (Quantikine ELISA kit, R&D Systems, Minneapolis, USA).

### Samples preparation for array analysis

Total RNA was isolated from FACS-purified primary β-cells using the RNeasy Mini Kit (Qiagen, Venlo, The Netherlands). The Aminoallyl antisense-cRNA (aa-cRNA) was obtained, coupled to either fluorophore indocarbocyanine (Cy3) or indodicarbocyanine (Cy5) (Amersham, Diegem, Belgium) and prepared for hybridization (10).

### Gene expression array data analysis

The first step of the GC-Robust MultiChip Average (GCRMA) algorithm is a background correction step were probe sequence information is used to estimate probe affinity to non-specific binding. The background adjusted probe intensities are then quantile normalized and converted to expression measures using the median polish procedure. These normalization and summarization steps are identical to those of the RMA (Robust MultiChip Average) method described by Irizarry (11;12). Detection calls (Present, Marginal or Absent) were produced using the Affymetrix standard method MAS5 implemented in the Bioconductor's "affy" package to remove data that is not reliably detected before further analysis. Only probesets declared "Present" in at least 2 out of 3 experiments of at least one experimental condition (control, IL-1β+IFN-γ or TNF-α+IFN-γ) in at least one time point, were considered for further analysis; using this filtering criteria we retained 15.772 probesets. Each treatment group (IL-1β+IFN-γ or TNF-α+IFN-γ) was compared at different time points (6h or 24h) to the corresponding untreated control group using a Welch's t-test based on robust estimators of central tendency (the median was considered as a robust alternative to the sample mean) and dispersion (the standard deviation was estimated on the basis of the inter-quartile range). Genes with a p-value $\leq$ 0.02 and at least a 1.5 median fold change between treatments (cytokines) and controls were considered as differentially expressed. The complete array data will be deposited, after publication of this article in the "Beta Cell Gene Bank" (http://www.t1dbase.org/cgi-bin/enter_bcgb.cgi).

**Exon array data analysis**

Exon expression values were calculated by running the ExonRMA algorithm from the ArrayAssist workflow of the extended probesets, with default parameters, adding a value of 16 for variance stabilization, before log2 transformation. Only probesets with a detection above background (DABG) (13) and p-value $\leq$ 0.05 in at least one sample (184.404 probesets) were considered. Transcripts with at least one differentially expressed exon (i.e. fold change in exon-level expression between treatment (cytokines) and control conditions greater than or equal to 2) were retrieved and their transcript-level expression values were generated using the same algorithm and associated parameters as those used for exon level summarization. Genes with a two fold or greater change in transcript-level expression between treatment and control conditions were considered as differentially expressed. A splice index (defined as the log of the ratio of exon-level expression over gene-level expression) was computed for each exon and those with a fold change in splice index value greater than or equal to 2 between treatment and control conditions were considered as differentially spliced. Transcripts with at least one differentially spliced exon were considered as showing differential splicing.

**In silico analysis of upstream gene sequences**

The 1 kb upstream sequences, starting from the transcription start site (TSS), of 6 hormone receptor genes (GLP-1R, Prlr, Gipr, Ghr, Vipr2 and Cckar ) and 9 Krebs cycle-related genes (Cs, Aco2, Idh3B, Idh3g, αkgdh, Suclg1, Sdhb, Fh1 and Mdh2) were retrieved from the ENSEMBL database (release 48, http://www.ensembl.org) together with a large set of background sequences (2 kb upstream sequences of the whole rat genome). The Clover program (14) was then used to scan through all vertebrate positional weight matrices (PWMs) of the TRANSFAC Professional Database (version 11.1) (15) to detect motifs that are statistically over-represented in the two sets of target sequences as compared to background sequences. The Clover method first calculates a raw score for each motif, indicating the strength of its presence in the target sequences. In a second step, for each raw score the program repeatedly selects random segments matched by length to the target sequences, from the background sequences (1000 randomizations), calculates raw scores for these fragments and uses these to estimate a p-value.

The p-value indicates the probability that the motif's presence in the target sequences is explained just by chance. Motifs were declared as significantly over-represented in the promoter sequences of the hormone receptor genes or in those of the Krebs cycle-related genes if their p-values were $\leq 0.05$ or $\leq 0.01$, respectively.

**RT-PCR and real-time RT-PCR**

After exposure to cytokines during time-course experiments, cells were harvested and reverse transcriptase reaction was performed using poly(A)+RNA (16). Confirmation of array results was done on cDNA from independent experiments from those used in the arrays studies. Semi-quantitative RT-PCR was done as described (17) for confirmation of alternative splicing induced by cytokines detected in the exon array analysis. Confirmation of microarray results of selected genes modified by cytokines was performed by real time RT-PCR using SYBR Green fluorescence on a LightCycler instrument (Roche Diagnosis, Manheim, Germany) by the standard curve method (18;19). The PCR amplification reactions and preparation of standards were performed as previously described (18). Expression values of the genes of interest were corrected by the expression values of the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and normalized by the higher value in the experimental series considered as 1. We have previously shown that cytokines do not modify GAPDH expression in insulin-producing cells (5;8;16) and these observations were confirmed in the present series of experiments (data not shown). Primers sequences and their respective PCR fragment lengths are shown in Table I, Supplementary Material.

**Immunofluorescence**

Cells were plated in poly-lysine coated cover slip plates (20.000/codition) and after experimental treatment fixed with 4% paraformeldehyde, permeabilized with 70% acetone + 30% methanol. Cells were then incubated for 1h with anti-p65 (Santa Cruz Biotechnology, Santa Cruz, CA-EUA) or, as a negative control, with anti-hemaglutinin (HA) (Roche Diagnostics, Mannheim-Germany) both at a 1:500 dilution. The secondary antibody used for visualization was anti-rabbit IgG conjugated with FITC (Jackson ImmunoResearch, Westgrove, PA-EUA), diluted at 1/200. Samples were examined by inverted fluorescence microscopy (Zeiss Axiovert 200, Oberkochen-Germany); NF-κB activation was confirmed by the presence of p65 in the nucleus and the percentage of positive cells was determined in at least 500 cells in each experimental condition by two observers, one of whom was unaware of samples identity.

**Promoter reporter assays**.

Rat purified β-cells were transfected as previously described (20) with pRL-CMV encoding Renilla luciferase (Promega Corp) and a luciferase promoter-reporter construct containing 6 hypoxia-responsive elements (6HRE – binding site for HIF-1α; plasmid kindly provided by Prof. C. Michiels, Université de Namur-Belgium). Luciferase activities were assayed after 12h cytokine treatment as previously described (20). Cobalt chloride ($CoCl_2$) was used as a positive control for HIF-1 activation at the concentration of 150μM.

***Supplementary Reference List***


1. Pipeleers, DG , in't Veld, PA, Van de Winkel, M, Maes, E, Schuit, FC, Gepts, W: A new in vitro model for the study of pancreatic α and β cells. *Endocrinology* 117:806-816, 1985
2. Rasschaert, J, Ladriere, L, Urbain, M, Dogusan, Z, Katabua, B, Sato, S, Akira, S, Gysemans, C, Mathieu, C, Eizirik, DL: Toll-like receptor 3 and STAT-1 contribute to double-stranded RNA+ interferon-γ-induced apoptosis in primary pancreatic β-cells. *J Biol Chem* 280:33984-33991, 2005
3. Ling, Z, Hannaert, JC, Pipeleers, D: Effect of nutrients, hormones and serum on survival of rat islet β-cells in culture. *Diabetologia* 37:15-21, 1994
4. Eizirik, DL, Mandrup-Poulsen, T: A choice of death-the signal-transduction of immune-mediated β-cell apoptosis. *Diabetologia* 44:2115-2133, 2001
5. Cardozo, AK, Kruhoffer, M, Leeman, R, Orntoft, T, Eizirik, DL: Identification of novel cytokine-induced genes in pancreatic β-cells by high-density oligonucleotide arrays. *Diabetes* 50:909-920, 2001
6. Flodstrom, M, Niemann, A, Bedoya, FJ, Morris, SM Jr, Eizirik, DL: Expression of the citrulline-nitric oxide cycle in rodent and human pancreatic β-cells: induction of argininosuccinate synthetase by cytokines. *Endocrinology* 136:3200-3206, 1995
7. Hoorens, A, Van de Casteele, M, Kloppel, G, Pipeleers, D: Glucose promotes survival of rat pancreatic β-cells by activating synthesis of proteins which suppress a constitutive apoptotic program. *J Clin Invest* 98:1568-1574, 1996
8. Kutlu, B, Cardozo, AK, Darville, MI, Kruhoffer, M, Magnusson, N, Orntoft, T, Eizirik, DL: Discovery of gene networks regulating cytokine-induced dysfunction and apoptosis in insulin-producing INS-1 cells. *Diabetes* 52:2701-2719, 2003
9. Green, LC, Wagner, DA, Glogowski, J, Skipper, PL, Wishnok, JS, Tannenbaum, SR: Analysis of nitrate, nitrite, and nitrate in biological fluids. *Anal Biochem* 126:131-138, 1982
10. Magnusson, NE, Cardozo, AK, Kruhoffer, M, Eizirik, DL, Orntoft, TF, Jensen, JL: Construction and validation of the APOCHIP, a spotted oligo-microarray for the study of β-cell apoptosis. BMC Bioinformatics 6:311, 2005
11. Irizarry, RA, Hobbs, B, Collin, F, Beazer-Barclay, YD, Antonellis, KJ, Scherf, U, Speed, TP: Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics* 4:249-264, 2003
12. Irizarry, RA, Bolstad, BM, Collin, F, Cope, LM, Hobbs, B, Speed, TP: Summaries of Affymetrix GeneChip probe level data. *Nucleic Acids Res* 31:e15, 2003
13. Affymetrix (2005) Exon array background Correction. Affymetrix Whitepaper. Available: http://www.affymetrix.com/support/technical/whitepapers/exon_background_correction_whitepaper.pdf. Accessed 15 July 2009.
14. Frith, MC, Fu, Y, Yu, L, Chen, JF, Hansen, U, Weng, Z: Detection of functional DNA motifs via statistical over-representation. *Nucleic Acids Res* 32:1372-1381, 2004
15. Matys, V, Kel-Margoulis, OV, Fricke, E, Liebich, I, Land, S, Barre-Dirrie, A, Reuter, I, Chekmenev, D, Krull, M, Hornischer, K, Voss, N, Stegmaier, P, Lewicki-Potapov, B, Saxel, H, Kel, AE, Wingender, E: TRANSFAC and its module TRANSCompel: transcriptional gene regulation in eukaryotes. *Nucleic Acids Res* 34:D108-110, 2006
16. Cardozo, AK, Ortis, F, Storling, J, Feng, YM, Rasschaert, J, Tonnesen, M, Van Eylen, F, Mandrup-Poulsen, T, Herchuelz, A, Eizirik, DL: Cytokines downregulate the

sarcoendoplasmic reticulum pump Ca2+ ATPase 2b and deplete endoplasmic reticulum Ca2+, leading to induction of endoplasmic reticulum stress in pancreatic β-cells. *Diabetes* 54:452-461, 2005

17. Cardozo, AK, Heimberg, H, Heremans, Y, Leeman, R, Kutlu, B, Kruhoffer, M, Orntoft, T, Eizirik, DL: A comprehensive analysis of cytokine-induced and nuclear factor-κB-dependent genes in primary rat pancreatic beta-cells. *J Biol Chem* 276:48879-48886, 2001
18. Kharroubi, I, Rasschaert, J, Eizirik, DL, Cnop, M: Expression of adiponectin receptors in pancreatic β-cells. *Biochem Biophys Res Commun* 312:1118-1122, 2003
19. Cardozo, AK, Proost, P, Gysemans, C, Chen, MC, Mathieu, C, Eizirik, DL: IL-1β and IFN-γ induce the expression of diverse chemokines and IL-15 in human and rat pancreatic islet cells, and in islets from pre-diabetic NOD mice. *Diabetologia* 46:255-266, 2003
20. Ortis, F, Pirot, P, Naamane, N, Kreins, AY, Rasschaert, J, Moore, F, Théatre, E, Verhaeghe, C, Magnusson, N, Chariot, A, Orntoft, T, Eizirik, DL: TNF-α and IL-1β induction of NF-κB and its downstream genes has a pro-apoptotic role in pancreatic β-cells. Diabetologia 51:1213-1225, 2008

**Table IS: Primer sequences and their respective PCR fragment lengths**

| Name | Forward | Reverse | lengths |
|---|---|---|---|
| CCL5 ST | 5'-GCTGCTTTGCCTACCTCTC-3' | 5'-ATCTATGCCCTCCCAGGAAT-3' | 302 bp |
| CCL5 RT | 5'-CCAGAGAAGAAGTGGGTTCA-3' | 5'-AGCAAGCAATGACAGGAAAG-3' | 104 bp |
| IRF1 ST | 5'-TGAAGCTGCAACAGATGAGG-3' | 5'-TCACTCAGGAGGGCAAGAAC-3' | 358 bp |
| IRF1 RT | 5'-CAAGGAGGAACCAGAGATCG-3' | 5'-CAGGGACCCAAATTATGGTG-3' | 165 bp |
| IRF7 ST | 5'-CCAGATGCGTGTTCCTGTAT-3' | 5'-GGCAAGTGCAAGGTGTACTG-3' | 384 bp |
| IRF7 RT | 5'-GCCCAAAACCCAGGTAGAT-3' | 5'-GCCCAAAACCCAGGTAGA-3' | 193 bp |
| CXCL10 ST | GAAGCACCATGAACCCAAGT | GCAAGTCTATCCTGTCCGCAT | 380 bp |
| CXCL10 RT | GGGTAAAGGGAGGTGGAGAG | GGGTAAAGGGAGGTGGAGAGA | 216 bp |
| MCP-1 ST | 5'-GAAACCAGCCAACTCTCACT-3' | 5'-GCATCACATTCCAAATCACA-3' | 780 bp |
| MCP-1 RT | 5'-TAGCATCCACGTGCTGTCTC-3' | 5'-TGCTGCTGGTGATTCTCTTG-3' | 122 bp |
| STAT-1 ST | 5'-CCTCTTCCAGCAGCTC-3' | 5'-TGAGTTCCGACACCTGCAACTGAA-3' | 596 bp |
| STAT-1 RT | 5'-ACTGCCAACTCAGCAC-3' | 5'-AGGTGGTCTCAAGGTCAATCACCA-3' | 149 bp |
| IL-15 ST | 5'-TCTTAACTGAGGCTGGCATC-3' | 5'-TTGTTAGAAGACAGAGTGCTG-3' | 304 bp |
| IL-15 RT | 5'-ATGTAAGATACGATCTGGAG-3' | 5'-AGTCATGTTACTGTACTCGTG-3' | 164 bp |
| CXCL9 ST | 5'-GGAGTTCGAGGAACCCTAGT-3' | 5'-CAAGGCACATTCCACTACAA-3' | 352 bp |
| CXCL9 RT | 5'-CAGAGCGCTTGTTGGTAAA-3' | 5'-CCTTGCTGAATCTGGGTCTA-3' | 152 bp |
| CXCL1 ST | 5'-TCCAACAGAGAGCACCATGGTC-3' | 5'-TCTCCATTACTTGGGGACAC-3' | 310 bp |
| CXCL1 RT | 5'-TCCAGAGTTTGAAGGTGATG-3' | 5'-AGCATCTTTTGGACAATCTTC-3' | 131 bp |
| Mafa ST | 5'-AGCTGGTGTCCATGTCAGTG-3' | 5'-CCGCCAACTTCTCGTATTTCTCC-3' | 264 bp |
| Mafa RT | 5'-AAGGAGGAGGTCATCCGACT-3' | 5'-TCTGGAGCTGGCACTTCTCG-3' | 128 bp |
| PDX-1 ST | 5'-CGCATGAAGTGGAAGAAAGAG-3' | 5'-TGTTATGGGACCGCTCAAGTT-3' | 601 bp |
| PDX-1 RT | 5'-GGTATAGCCAGCGAGATGCT-3' | 5'-TCAGTTGGGAGCCTGATTCT-3' | 153 bp |
| Isl-1 ST | 5'-ACAAGAAACGCAGCATCATG-3' | 5'-ACCATGGGAGTTCCTGTCAT-3' | 463 bp |
| Isl-1 RT | 5'-AGTGCGGACTGTGCTCAAC-3' | 5'-ATCTGGGAGCTGAGAGGACA-3' | 91 bp |
| Idh3b ST | 5'-GGTGCCTTTCCTGTGACAAT-3' | 5'-TCCCATAGAGATTGGGCATC-3' | 685 bp |
| Idh3b RT | 5'-GGTGTCATTGAGTGCCTGAA-3' | 5'-AAGAACAGCCCATCTCCAAG-3' | 149 bp |
| αKgdh ST | 5'-GCCAATGTCATCAGGAAGGA-3' | 5'-GCTTCTGCTTACGGATCTGC-3' | 649 bp |
| αkgdh RT | 5'-TAGAGGCTGCTGACCCTGTA-3' | 5'-TCTGTGGTGAAGCCAATCTG-3' | 223 bp |
| mdh ST | 5'-GACCAGCCCATCATTCTTGT-3' | 5'-ATCAGAGATTACGCCCATCG-3' | 726 bp |
| mdh RT | 5'-GACCTACTGAAAGCCAACGT-3' | 5'-TTGTGGTCCAATCGAGTCAG-3' | 185 bp |
| Fh1 ST | 5'-CAATAATGAAGGCCGCAGAT-3' | 5'-AAAGGCAAACCTGTGAGTGC-3' | 559 bp |
| Fh1 RT | 5'-TTACAGAAGCTGCACGATGC-3' | 5'-GAAGCCGATCCTGGTGTTTA-3' | 237 bp |
| Suclg2 ST | 5'-TGAAGATTGACGCCACTCAG-3' | 5'-AACAGCTTTCTTGGCTGCAT-3' | 581 bp |
| Suclg2 RT | 5'-GTCAACTGTGCCATCATTGC-3' | 5'-TTCTGAGCCTCCTGGACATT-3' | 110 bp |
| Sdhb ST | 5'-GCAGCTCCCAGAATCAAAAC-3' | 5'-CTTGTCTCCGTTCCACCAGT-3' | 513 bp |
| Sdhb RT | 5'-GCAGGATCGACACAGACCTT-3' | 5'-GCACTCATACAATCCGTCCA-3' | 215 bp |
| Cs ST | 5'-TACCTAAGGAGCAGGCCAGA-3' | 5'-TTTCACTGTTGAGGGCTGTG-3' | 414 bp |
| Cs RT | 5'-CACATGCCCACAGAGGAACA-3' | 5'-AATTGTCCAGCATGGTGACC-3' | 95 bp |
| Aco 2 ST | 5'-TGGACCCTTTACCCCTGACT-3' | 5'-GTATGTGTCCTGCCCTGGAT-3' | 583 bp |
| Aco 2 RT | 5'-CACGGACTCAAGTGCAAGTC-3' | 5'-GATTCCACCCACATCCCTTA-3' | 111 bp |
| ATF4 ST | 5'-TCCTGAACAGCGAAGTGTTG-3' | 5'-AGGGAAGAGGCTGCAAGAAT-3' | 471 bp |
| ATF4 RT | 5'-GTTGGTCAGTGCCTCAGACA-3' | 5'-CATTCGAAAGCAAGCATCGA-3' | 109 bp |
| GlpR1 ST | 5'-ATCCACCTGAACCTGTTTGC-3' | 5'-CTTGGCTATCACGATGCAGA-3' | 468 bp |
| GlpR1 RT | 5'- GCTGCCCTCAAGTGGATGTA-3' | 5'-ATGAGCAGGAACACCAGTCG-3' | 108 bp |
| PrlR ST | 5'-GCAGAAGAGTGGGAGATCCA-3' | 5'-CCAGCAAGTCCTCACAGTCA-3' | 406 bp |
| PrlR RT | 5'-TGATTATGGTCTGGGCAGTG-3' | 5'-TCTCCAGCAGATGGGTATCA-3' | 108 bp |
| GhR ST | 5'-GAATGGAAAGAATGCCCTGA-3' | 5'-TCCGTTGTCTGGATCTCACA-3' | 418 bp |
| GhR RT | 5'-TGGTGATTTGTTGGACGAAA-3' | 5'-ATCCCAGGCAAACTGATGTT-3' | 105 bp |
| CckaR ST | 5'-GCCAGCACTTGGTAGAAAGC-3' | 5'-TGGTAAAGGAGAGGCACCAG-3' | 540 bp |
| CckaR RT | 5'-CCAACCTGCTCAAGGATTTC-3' | 5'-TGAAGGTGGAAACGCTCAC-3' | 90 bp |
| HIF1α ST | 5'-TCAAGTCAGCAACGTGGAAG-3' | 5'-GATTCAGTGCAGGATCAGCA-3' | 861 bp |
| HIF1α RT | 5'-GCTTGGTGCTGATTTGTGAA-3' | 5'-TATCGAGGCTGTGTCGACTG-3' | 93 bp |
| GAPDH ST | 5'-ATGACTCTACCCACGGCAAG-3' | 5'-TGTGAGGGAGATGCTCAGTG-3' | 930 bp |
| GAPDH RT | 5'-AGTTCAACGGCACAGTCAAG-3' | 5'-TACTCAGCACCAGCATCACC-3' | 136 bp |
| iNOS exon 1-10 | 5'-TCAGGCTTGGGTCTTGTTAG-3' | 5'-CGCTGTGTGTCACAGAAGTC-3' | 1237 bp |
| Ass exon 1-5 | 5'-CCTGTGCTTATAACCCTGGA-3' | 5'-TCCACTTGTTTGCGAGCTAT-3' | 453 bp |
| NF-κB2 exon 21-22 | 5'-GGGTCTATTGGAAGCCCTTGT-3' | 5'-AGGGACTGGCGTGACATT-3' | 372 bp |
| | | | |

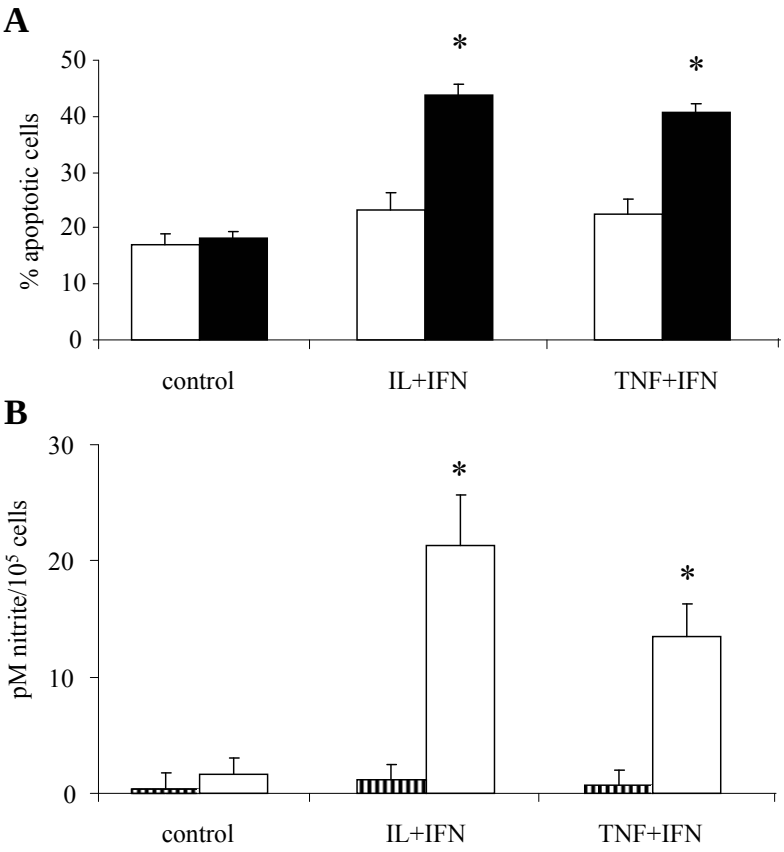
ST: Standard curve; RT: Real time RT-PCR

**Suppl. Table II.** siRNAs used to knockdown gene/protein expression.

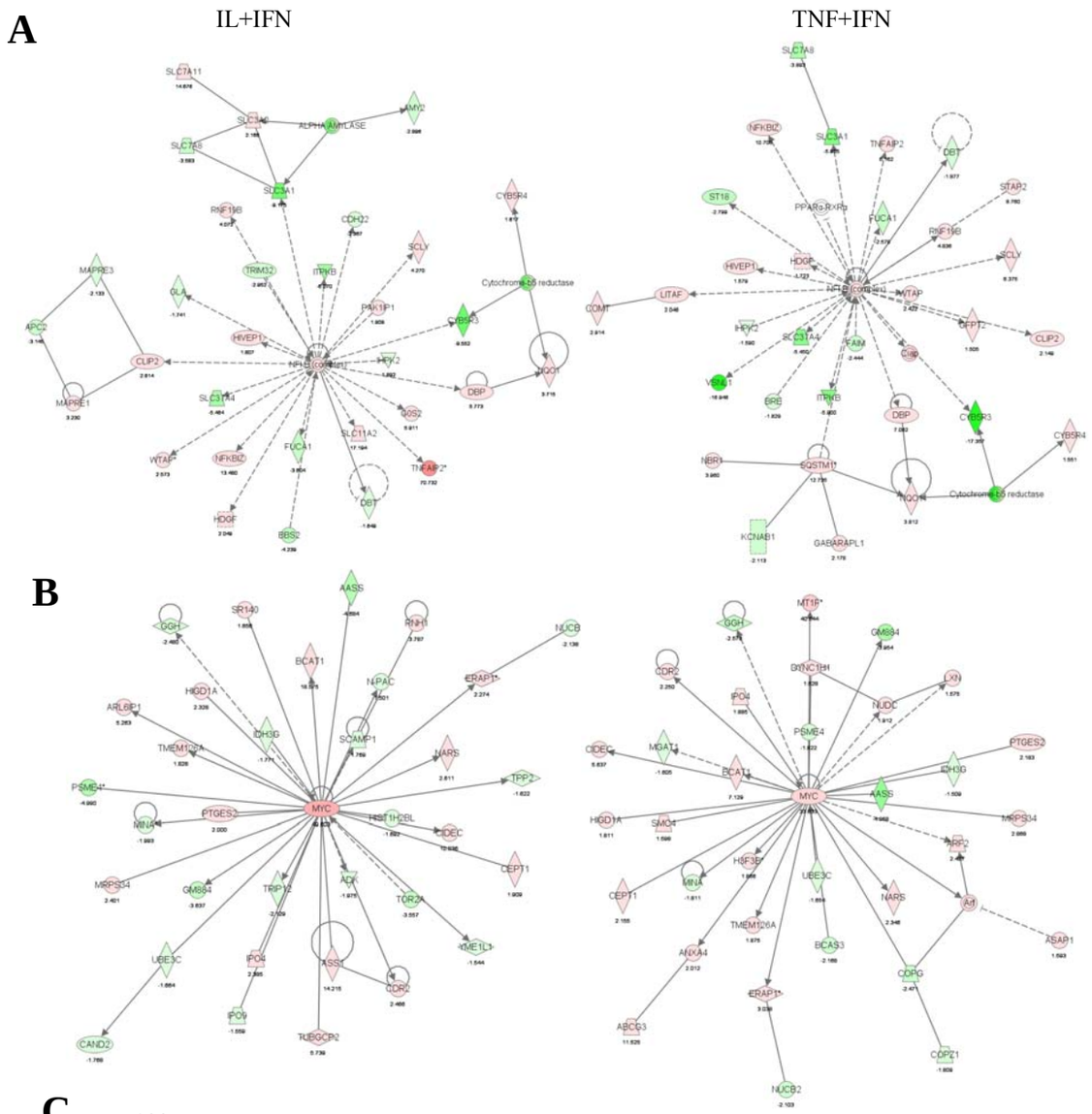
| siRNA | Supplier | Sequence |
|---|---|---|
| Allstars Negative Control siRNA | Qiagen, Venlo, Netherlands | |
| ON-TARGETplus rat HIF-1 SMARTpool® | Thermo Scientific, Chicago, USA | seq #1 5'-UGAGAGAAAUGCUUACACA-3' |
| | | seq #2 5'-GGAAACGAGUGAAAGGAUA-3' |
| | | seq #3 5'-UUACUGAGUUGAUGGGUUA-3' |
| | | seq #4 5'-CUGAUAACGUGAACAAAUA-3' |
| rat ATF3 | Invitrogen, Carlsbad, USA | 5'-UGGAGAGUGUGAAUGCCGAACUGAA-3' |
| rat ATF4 | Invitrogen, Carlsbad, USA | 5'-UCGUGGUUCUCGACCCAAACCUUAU-3' |
| Silencer® Select siRNA rat IRF7 | App. Biosystems, Austin, USA | 5'-CCAAUAGUCUCUACGAUGAtt-3' |
| ON-TARGETplus rat IRF7 SMARTpool® | Thermo Scientific, Chicago, USA | seq #1 5'-CACUAUGGGCCCUGCGAAU-3' |
| | | seq #2 5'-GGUCAGUAGUGGCCGGUAC-3' |
| | | seq #3 5'-CCAAUAGUCUCUACGAUGA-3' |
| | | seq #4 5'-UGGAAGCAUUUCAGCCGUA- 3' |

**(See individual files for Supplementary Tables III – Table XII.)**

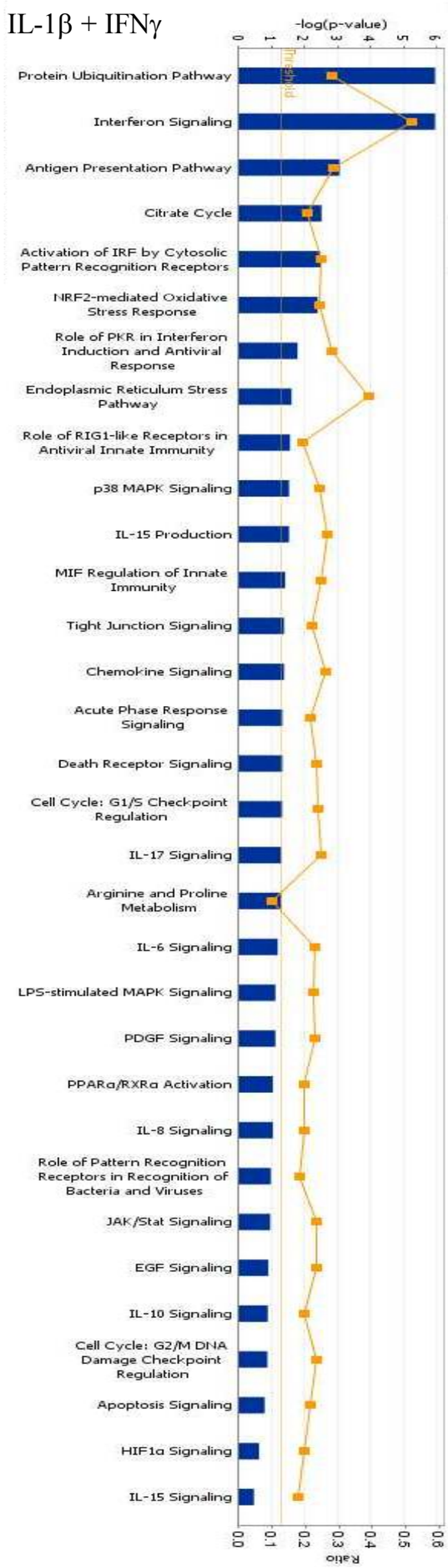
**Suppl. Figure 1: Effects of cytokine exposure on the viability and NO formation in rat β-cells.** FACS-purified rat β-cells were exposed or not (control) to IL-1β+IFN-γ (IL+IFN) or to TNF-α+IFN-γ (TNF+IFN) for 6h (vertical striped bars), 24h (white bars) or 72h (black bars). **A**: viability assay; **B**: Nitrite accumulation in the medium from cells used in the microarray analysis. Results are the mean ± SEM of 3-6 experiments. **p* < 0.05 vs control cells.



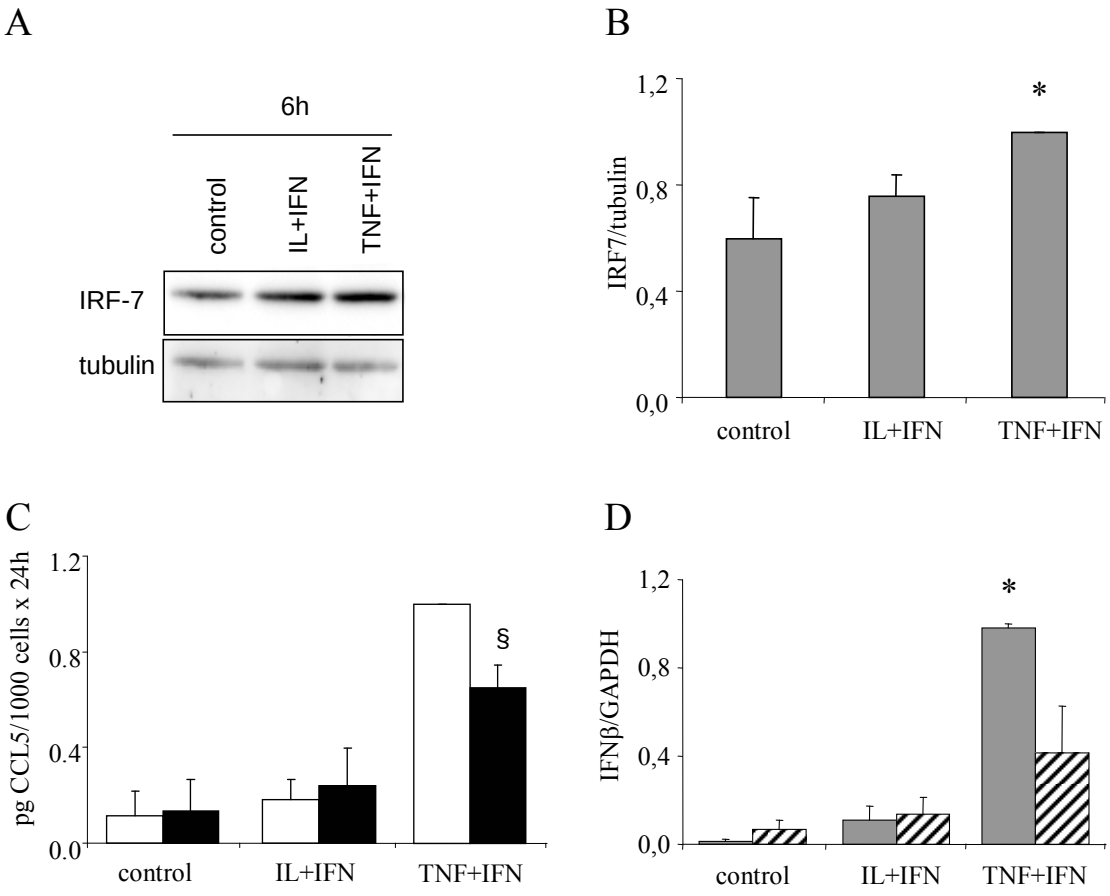
**Suppl. Figure 2:** Differential usage of gene networks induced by IL-1β+IFN-γ or TNF-α+IFNγ. **A-B:** Gene networks regulated by the transcription factor NF-κB (**A**) or c-Myc (**B**) after IL-1β+IFNγ (IL+IFN) or TNF-α+IFNγ (TNF+IFN). Genes with up-regulated expression are colored in pink/red and genes with down-regulated expression are colored in green. The intensity of the colors reflects the intensity of regulation after 24h of cytokine treatment. Geometrical symbols represent gene products function: enzymes (vertical diamond), growth factors (square), ion channels (rectangle), kinases (inverted pyramid), peptidases (horizontal diamond), transcription factors (elliptical), transporters (trapezoid isosceles) and others (circle). Solid lines indicate direct interactions (gene or gene products have direct physical interactions), whereas dotted lines indicate indirect interactions. The analysis was done by the use of IPA 5.5 software **C:** Temporal analysis of IL-1β or TNF-α-induced NF-κB nuclear localization in rat primary β-cells. Cells were exposed to IL-1β (white bars) or TNF-α (black bars) or left untreated (vertical striped bars) at the indicated time points. Anti-p65 antibody was used to measure NF-κB translocation to the nucleus. Results are mean of 4 independent experiments. * $p < 0.05$ IL-1β vs. TNF-α, paired *t*-test.



**Suppl. Figure 3:** Canonical Pathways preferentially regulated by IL-1β+IFN-γ or TNF-α+IFN-γ at 24h. The figure shows 32 selected pathways classified by IPA 5.5 software according to the level of significance (blue bars). Selection of the pathways was based on higher statistic significance, ratio between modified genes over total number of genes present in the pathway (yellow line) and the potential importance of the pathway for cytokine-modified β-cell function and survival.

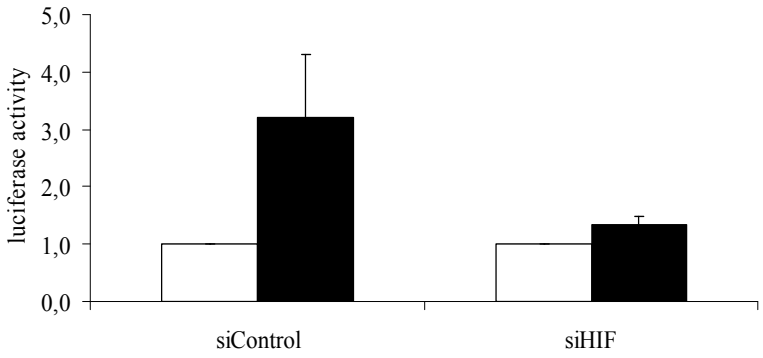



**Suppl. Figure 4: Differential regulation of protein expression by TNF-α+IFN-γ or IL-1β+IFN-γ in β-cells.** A-B: Western blot for IRF-7 in primary rat β-cell after cytokine treatment. FACS purified rat β-cells were left untreated (control) or exposed to IL-1β+IFN-γ (IL+IFN) or TNF-α+IFN-γ (TNF+IFN) for 6h. A: Representative figure of 3 independent experiments. B: Quantification of 3 independent Western blots for IRF-7 corrected by tubulin at 6h (grey bars). * $P \leq 0.05$ vs IL-IFN at the same time point by paired *t-test*. C: Effects of cytokines on CCL5 secretion to the medium by primary β-cells. FACS purified rat β-cells were transfected with siRNA control (white bars) or siRNA against IRF-7 (black bars) and exposed or not (control) to IL-1β+IFN-γ (IL+IFN) or to TNF-α+IFN-γ (TNF+IFN) for 24h. Results are mean ± SEM of 4 independent experiments. §$P \leq 0.05$ vs siControl at the same time point and treatment. CCL5 was measured in the cell supernatant by enzyme-linked immunosorbent assay. D: Real-time PCR for mRNA expression of IFN-β. FACS purified rat β-cells were left untreated (control) or exposed to IL-1β+IFN-γ (IL+IFN) or TNF-α+IFN-γ (TNF+IFN) for 6 (grey bars) or 24h (diagonal striped bars). * $P \leq 0.05$ vs IL-IFN at the same time point by paired *t-test*.

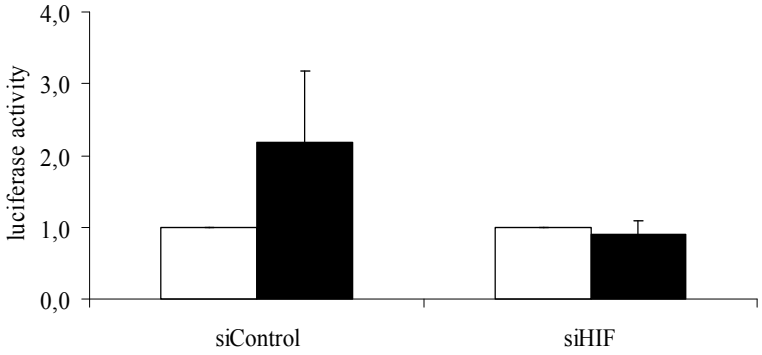


**Suppl. Figure 5: HIF-1α induction in β-cells. A-B:** Luciferase reporter assay of HIF-1α activation by cytokines. Dispersed islets cells (A) or INS-1E cells (B) were co-transfected with an HRE luciferase reporter gene, the internal control pRL-CMV and the siHIF, then left untreated (white bars) or treated for 24h with IL-1β+IFN-γ (black bars). Results are normalized for *Renilla* luciferase activity and are means ± of 3-4 experiments. * $p \leq 0.05$ *vs*. untreated cells. **C:** Western blot for P-AKT protein from FACS purified β-cells untreated or exposed to IL-1β+IFN-γ for 6 or 24h. The figure is representative of 3 independent experiments.

A




B




C