The Protective Action of Metformin against Pro-Inflammatory Cytokine-Induced Human Islet Cell Damage and the Mechanisms Involved

Laura Giusti 1,†, Marta Tesi 2,†, Federica Ciregia 2,3, Lorella Marselli 2 4, Lorenzo Zallocco 4 5,6, Mara Suleiman 2, Carmela De Luca 2, Silvia Del Guerra 2, Mariachiara Zuccarini 5,6, Marco Trerotola 5,6, Decio L. Eizirik 7, Miriam Cnop 7, Maria R. Mazzoni 4, Piero Marchetti 2,*, Antonio Lucacchini 2 8 and Maurizio Ronci 5,8 9

1 School of Pharmacy, University of Camerino, 62032 Camerino, Italy
2 Department of Clinical and Experimental Medicine, University of Pisa, 56126 Pisa, Italy
3 Laboratory of Rheumatology, GIGA Research, CHU de Liège, University of Liège, 4000 Liège, Belgium
4 Department of Pharmacy, University of Pisa, 56126 Pisa, Italy
5 Center for Advanced Studies and Technologies (CAST), University of Chieti-Pescara, 66100 Chieti, Italy
6 Department of Medical, Oral and Biotechnological Sciences, University “G. d’Annunzio” of Chieti-Pescara, 66100 Chieti, Italy
7 ULB Center for Diabetes Research, Université Libre de Bruxelles, 1070 Brussels, Belgium
8 Department of Pharmacy, University “G. d’Annunzio” of Chieti-Pescara, 66100 Chieti, Italy
* Correspondence: piero.marchetti@med.unipi.it
† These authors contributed equally to this work.

Abstract: Metformin, a drug widely used in type 2 diabetes (T2D), has been shown to protect human β-cells exposed to gluco- and/or lipotoxic conditions and those in islets from T2D donors. We assessed whether metformin could relieve the human β-cell stress induced by pro-inflammatory cytokines (which mediate β-cells damage in type 1 diabetes, T1D) and investigated the underlying mechanisms using shotgun proteomics. Human islets were exposed to 50 U/mL interleukin-1β plus 1000 U/mL interferon-γ for 48 h, with or without 2.4 µg/mL metformin. Glucose-stimulated insulin secretion (GSIS) and caspase 3/7 activity were studied, and a shotgun label free proteomics analysis was performed. Metformin prevented the reduction of GSIS and the activation of caspase 3/7 induced by cytokines. Proteomics analysis identified more than 3000 proteins in human islets. Cytokines alone altered the expression of 244 proteins (145 up- and 99 down-regulated), while, in the presence of metformin, cytokine-exposure modified the expression of 231 proteins (128 up- and 103 downregulated). Among the proteins inversely regulated in the two conditions, we found proteins involved in vesicle motility, defense against oxidative stress (including peroxiredoxins), metabolism, protein synthesis, glycolysis and its regulation, and cytoskeletal proteins. Metformin inhibited pathways linked to inflammation, immune reactions, mammalian target of rapamycin (mTOR) signaling, and cell senescence. Some of the changes were confirmed by Western blot. Therefore, metformin prevented part of the deleterious actions of pro-inflammatory cytokines in human β-cells, which was accompanied by islet proteome modifications. This suggests that metformin, besides use in T2D, might be considered for β-cell protection in other types of diabetes, possibly including early T1D.

Keywords: β-cell; cytokines; metformin; proteomics; label-free shotgun analysis

1. Introduction

Diabetes mellitus (DM) is a disorder of the metabolism of carbohydrate, fat, and protein, due to the interplay of genetic and environmental factors [1,2]. It is characterized by an absolute or relative shortage of insulin production and secretion by the pancreatic islet β-cells [1,3,4]. In 2021 there were 537 million people (age 20–79 yrs) with DM,
which is expected to increase to 643 million by 2030 and 783 million by 2045 [2]. Morbidity and mortality in diabetic subjects are high, mainly due to the acute metabolic and chronic vascular complications of the disease, and it has been calculated that approximately 6.7 million diabetic adults died in 2021 [2]. In parallel, the direct costs of diabetes grew to USD 966 billion in 2021 [2]. Therefore, better strategies to prevent and treat this disease are needed.

Type 2 diabetes (T2D) is the most common form of DM, representing approximately 90% of all cases [1–4]. Several drugs are used to treat T2D, and metformin is the most widely employed [5,6]. Metformin is derived from galegine, a natural component of Galega Officinalis, a plant used in herbal medicine in medieval Europe and that was introduced into clinical use for the treatment of T2D in the 1950s [7]. Although its molecular mechanisms of action remain to be fully elucidated, metformin has been proven to be a safe and effective therapy, and it is now recommended as the first-line pharmacological treatment against T2D [8]. Metformin reduces blood glucose levels by decreasing hepatic glucose production, modifying the gut microbiome, and enhancing GLP-1 secretion [9–12]. In addition, the drug has anti-inflammatory properties, as indicated by its reduction of the neutrophil to lymphocyte ratio in subjects with T2D, anti-oxidative stress action, direct inhibitory effects on NF-kB signaling, and suppression of inflammatory cytokines in non-diabetic individuals [13–15].

Cytokines are small proteins produced by immune cells and other cell types that may have pro-inflammatory or anti-inflammatory effects, and which act via autocrine, paracrine, and/or endocrine mechanisms. A large body of evidence shows that pro-inflammatory cytokines (locally produced by immune cells in the course of insulitis) are involved in the pathogenesis of type 1 diabetes (T1D) [16–21]. At the level of the β-cells, they contribute to β-cell dysfunction and/or death during the early (particularly type 1 interferons, such as interferon-a (IFN-a)) and late (particularly interleukin-1b (IL-1b) and interferon-g (IFN-g)) phases of insulitis in type 1 diabetes.

Interestingly, a few studies have shown that metformin could have direct protective effects on β-cells under metabolic stress, including non-diabetic and T2D human islet cells [22–30]. However, it is not known whether metformin directly protects human β-cells against the damage induced by pro-inflammatory cytokines, nor the mechanisms possibly involved. Previous studies have indicated that the drug could shelter chondrocytes from IL-1β injury [31], reduce cytokine production in the cardiac muscle following ischemia-reperfusion [32], and limit the damage induced by lipopolysaccharide exposure in human bronchial epithelial cells [33].

In the present study we evaluated if metformin can defend human islet cells from IL-1β + IFN-γ-induced dysfunction and death. The mechanisms possibly involved were investigated at the proteome level with the use of shotgun proteomics, a bottom-up technique that enables comprehensive protein identification and profiling [34]. Although different tissues and cell types have been extensively evaluated using this approach [35,36], shotgun proteomics have been used to analyze pancreatic islets, the key tissue in diabetes pathogenesis [37–39], in only a few studies [40–42].

We show that, in our experimental conditions, metformin was able to shield isolated human islets from part of the insults induced by the tested cytokines, which was associated with several changes at the proteomic level, with the involvement of pathways mainly regulating inflammation and oxidative stress.

2. Methods

2.1. Human Pancreatic Islets

Isolated islets were prepared by enzymatic digestion and gradient purification from the pancreas of 14 multiorgan donors (age: 71 ± 9 years; 5M/9F; BMI: 26 ± 3 kg/m²) [43], with written consent by next-of-kin. Glands that were not suitable for clinical purposes were processed [43,44] with the approval of the local Ethics Committee (#2615 of 15 January 2014). We selected donors without a known history of diabetes. Following isolation, islets
were cultured in M199 medium (Euroclone SpA, Milan, Italy) containing 5.5 mM glucose, supplemented with 10% (v/v) adult bovine serum, 100 U/mL penicillin, 100 µg/mL streptomycin, 50 µg/mL gentamicin, and 750 ng/mL amphotericin B (all from Sigma-Aldrich, St. Louis, MO, USA) at 37 °C in a CO₂ incubator. For the purpose of the present study, approximately 1000 islets were incubated with cytokines (50 U/mL IL-1β, 1000 U/mL IFN-γ) for 48 h, in the presence and absence of metformin (2.4 µg/mL) (Sigma-Aldrich). This is a therapeutic concentration of the drug, which has been used in our laboratory previously [28,29]. The cytokine concentrations were based on those used by us and others in previous experiments [17,45–47]. Afterwards, isolated islets were used for functional, survival, and proteomics analyses, as described below.

2.2. Insulin Secretion Studies

Insulin release experiments were conducted as previously described [43,48,49]. After 45 min pre-incubation at 3.3 mM glucose, batches of 15 handpicked islets were challenged acutely (45 min) with 3.3- and 16.7-mM glucose. Then the islets were subjected to acid-alcohol extraction for insulin content measurement, as previously reported [43,48,49]. Insulin was quantified using a radioimmunometric assay (DIAsource ImmunoAssays S.A., Nivelles, Belgium). Insulin release was expressed as a percentage of the total insulin content. Insulin stimulation index was calculated as the ratio of insulin release at 16.7 mM glucose over the release at 3.3 mM glucose.

2.3. Caspase 3/7 Activity Assay

A Caspase-Glo® 3/7 assay kit (Promega Corporation, Madison, WI, USA) was used to detect caspase 3/7 activity, as described in [50,51]. Briefly, batches of 10 size-matched islets were seeded in a white solid 96-well plate, in a total volume of 100 µL/well. Then 100 µL of caspase 3/7 reagent, a solution containing luciferase and a tetrapeptide substrate linked to aminoluciferin, was added to each well and incubated for 1 h at room temperature. Following caspase cleavage of substrate, aminoluciferin was released and processed by luciferase, resulting in the production of light. Luminescence was recorded with a FLUOStar Omega microplate reader (BMG Labtech, Ortenberg, Germany).

2.4. Protein Extraction from Human Pancreatic Islets

The proteomic analysis was performed with islet preparations obtained from three different multiorgan donors (representing the biological replicates). Protein extraction from human pancreatic islets was performed as previously described [52]. Briefly, isolated islets were collected and washed twice with PBS (37 °C). Cells were suspended in the rehydration solution (7 M urea, 2 M thiourea, 4% CHAPS, 60 mM dithiothreitol (DTT), 0.002% bromophenol blue) containing 50 mM NaF, 2 mM Na₃VO₄, 1 µL/10⁶ cells of protease inhibitors, 1 µM trichostatin A, and 10 mM nicotinamide. After stirring and sonication (4 s, 5 times) cells were allowed to rehydrate for 1 h at room temperature (RT) with occasional stirring. Thereafter, the solution was centrifuged at 17,000× g for 5 min at RT. The protein concentration of the resulting supernatant was determined using the Bio-Rad RC/DC-protein assay (Bio-Rad). BSA was used as a standard.

2.5. Protein Fractionation

For shotgun analysis [53], technical triplicate experiments were performed on each of the three human islet preparations. For each preparation, three different conditions were analyzed, i.e., control islets, cytokine alone treatment, and cytokine plus metformin treatment. For this purpose, approximately 1000 human islets were treated as described above, and protein extracts were processed as follows: aliquots (40 µg of proteins) were loaded onto 12% acrylamide resolving gel and subjected to 1D-electrophoresis, as previously performed by us and others [43,54]. After protein staining using Coomassie blue R-250, 16 gel bands, matched for each lane, were excised and washed twice with wash buffer (25 mM NH₄HCO₃ in 50% acetonitrile). Afterwards, proteins were reduced with...
10 mM dithiothreitol (45 min, 56 °C) and alkylated with 55 mM iodoacetamide (30 min at RT in the dark). After two washes with the washing buffer, protein bands were completely dried in a centrivap vacuum centrifuge. Then the dried pieces of gel were rehydrated for 30 min at 4 °C in a porcine trypsin (Promega, Madison, WI, USA) solution (3 ng/µL in 100 mM NH₄HCO₃) and incubated overnight at 37 °C. The reaction was quenched by adding 10% trifluoroacetic acid. The samples were stored at −20 °C before being analyzed by LC-MS/MS.

2.6. Shotgun Label Free Analysis

The resulting peptides, 48 samples for each subject (16 controls, 16 treated with cytokines, and 16 treated with cytokines + metformin), were grouped by band and analyzed in technical triplicates using LC-MS/MS using a Proxeon EASY-nLCII (Thermo Fisher Scientific, Milan, Italy) chromatographic system coupled to a Maxis HD UHR-TOF (Bruker Daltonics GmbH, Bremen, Germany) mass spectrometer equipped with a nanoESI spray source. Peptides were loaded on an EASY-Column C18 trapping column (2 cm L, 100 µm I.D, 5 µm ps, Thermo Fisher Scientific) and subsequently separated on an Acclaim PepMap100 C18 (75 µm I.D., 25 cm L, 5 µm ps, Thermo Fisher Scientific) nano scale chromatographic column. The flow rate was set to 300 nL/min, and the gradient (mobile phase A: 0.1% formic acid in H₂O) was from 3 to 35% of mobile phase B (1% formic acid in acetonitrile) in 80 min, followed by 35 to 45% in 10 min and from 45 to 90% in 11 min. The mass spectrometer was operated in positive ion polarity and Auto MS/MS mode (data dependent acquisition—DDA), using N₂ as a collision gas for CID fragmentation. Precursors in the range of 350 to 2200 m/z (excluding 1220.0–1224.5 m/z) with a preferred charge state +2 to +5 (excluding singly charged ions) and absolute intensity above 4706 counts were selected for fragmentation in a maximum cycle time of 3 s. After acquiring one MS/MS spectrum, the precursors were actively excluded from selection for 30 s. Isolation width and collision energy for MS/MS fragmentation were set according to the mass and charge state of the precursor ions (from 3 to 9 Da and from 21 eV to 55 eV). In-source reference lock mass (1221.9906 m/z) was acquired online throughout the runs. Altogether, 432 instrumental runs were performed. Each raw data file was converted to mzXML format and submitted to LFQ processing (see below).

2.7. Raw Data Processing and Quantitative Analysis

Raw mass spectrometry data were analyzed using the PEAKS® Studio 7.5 software using the “correct precursor only” option. Spectra were matched against the neXtProt database (including isoforms as of June 2017; 42,151 entries), and the false discovery rate (FDR) was set to 0.1% at the peptide-spectrum matches (PSM) level. The post-translational modification (PTM) profile was set as follows: fixed cysteine carbamidomethylation (∆Mass: 57.02), variable methionine oxidation (∆Mass: 15.99), and glutamine and asparagine deamidation (∆Mass: 0.98). Non-specific cleavage was allowed to one end of the peptides, with a maximum of 2 missed cleavages and trypsin enzyme specificity. The highest error mass tolerances for precursors and fragments were set at 10 ppm and 0.05 Da, respectively. After processing every single raw data point, the label free quantification (LFQ) tool of PEAKS Studio was used to detect differentially expressed proteins. Parameters for LFQ were set as follows: quantification type as label free quantification; mass error tolerance, 10.0 ppm; retention time shift tolerance, 2.0 min; FDR threshold, 0.5%. The nine samples for each of the sixteen slices were allotted to 3 groups corresponding to Ctrl, Cyt, and Cyt + Met. For quantitative analysis, the significance threshold at the protein level was set to ≥ 20−10lgP with a fold change ≥2.0. Sixteen lists of differentially expressed proteins were obtained for each subject.

2.8. Pathway Analysis

Gene ontology and pathway analyses of differentially expressed proteins were performed with Metascape v3.5 (https://metascape.org/, accessed on 7 July 2022) [55] and
Ingenuity Pathway Analysis (IPA, QIAGEN Redwood City, www.qiagen.com/ingenuity, Build version: 321,501 M, Content version: 21249400, accessed on 7 July 2022), respectively. IPA core analysis provides not only gene functional annotation, canonical pathway, and network discovery, but also estimates the status of upstream regulators and downstream effects associated with canonical pathways, diseases, and functions. The upstream regulator analysis highlights the expected effects between the transcriptional regulators and their target genes [56]. The predicted activation or inhibition of each transcriptional regulator is inferred by the z score, which in turn is derived from the protein ratios in the dataset (z scores >2.0 indicate that a molecule is activated, whereas z scores < −2.0 indicate the inhibition of target molecules). SwissProt accession numbers with corresponding ratios were imported into the software, and the analysis was performed selecting only direct relationships among genes and molecules in all species and confidence settings were set to high predicted or experimentally observed. An IPA comparison analysis between the results of the different sections for every condition was also performed.

2.9. Western Blot

Western blot (WB) was performed as described [52], in order to confirm the shotgun results for ERAP2 and IFI30. Aliquots of protein samples (20 µg and 50 µg for IFI30 and ERAP2, respectively) were mixed with Laemmly solution, run in 4–15% polyacrylamide gels (Mini-PROTEAN® Precast Gels, Biorad) using a mini-Protean Tetracell (Biorad), and transferred onto nitrocellulose membranes (0.2 µm) using a Trans-Blot Turbo transfer system (Biorad), as described [52]. Anti-ERAP2 mouse monoclonal antibody (R&D Systems, Inc, Minneapolis, MN, USA) was used at 1:500 dilution, whereas anti-IFI30 mouse monoclonal antibody (sc-393507, Santa-Cruz Biotechnology, Inc, Dallas, TX, USA) was diluted 1:500. β-actin was used as a reference, and the anti-β-actin mouse monoclonal antibody (1:1000 dilution) (Merck group, De) was applied. HRP-goat anti-mouse secondary antibody was used at 1:10,000 dilution. Immunoblots were developed using the enhanced chemiluminescence detection system (ECL). Chemiluminescent images were acquired using LAS4010 (GE Health Care). The optical density (OD) of specific immunoreactive bands was quantified using Image Quant-L software (GE Health Care) and normalized by β-actin.

2.10. Statistical Analysis

Data are expressed as means ± SEM. The ANOVA test followed by Tukey correction was applied to assess the difference between groups in the insulin secretion and caspase activation experiments. A p value less than 0.05 was considered statistically significant. For proteomics analyses, we used the results generated with the islet preparations obtained from three different multorgans donors (biological replicates), with peptides from each fraction analyzed in technical triplicates. The false discovery rate (FDR) was set to 0.1% at the peptide–spectrum matches (PSM) level, resulting in an average protein FDR lower than 1.0%. Expression analysis for the relative abundance of identified proteins was performed at the band-slice level using the label-free quantification module PEAKS-Q, part of PEAKS Studio v. 7.5. This quantification method is based on the MS1 ion peak intensity of the extracted chromatograms of peptides detected in multiple samples and applies an expectation-maximization algorithm to detect and resolve overlapping features. A high-performance retention time alignment algorithm was also used to align features of the same peptide from multiple samples. The significance of the LFQ proteomics data provided directly from the software PEAKS Studio was calculated using the PEAKS Q method, which is similar to the significance B, as previously defined [57]. Briefly, protein ratios are calculated as the median of peptide ratios, minimizing the effect of outliers and normalizing the protein ratios, to correct for unequal protein amounts. An outlier significance score for log protein ratios is computed and a p-value for detection of significant outlier ratios is defined. Peptide ratios are calculated using the XIC of three different peptides. The differentially expressed proteins were calculated for each band using 0.5% PSMs FDR, and the resulting ID lists were filtered, considering only peptides confidently identified in at
least 1 sample with significance $\geq 20$ ($-10\lg P$) and quality factor $\geq 0.5$, and by considering only proteins identified with a significance $\geq 20$ and fold change $\geq 2$. The lists of the resulting dysregulated proteins of each band–slice were merged and manually curated to remove proteins identified in multiple adjacent bands, non-distinguishable isoforms, and keratins.

3. Results

3.1. Effects of Metformin on Cytokine-Induced Damage

The first aim of the present work was to assess whether metformin could protect human islets from cytokine toxicity, as previously observed for lipotoxic and glucotoxic damage [28,30]. The insulin content in cytokine-exposed islets was lower than in control cells and was not significantly modified by the presence of metformin (Figure 1A). As expected, the insulin stimulation index in response to glucose was significantly reduced after cytokine treatment (Figure 1B). However, with metformin added to the cytokines, the $\beta$-cell responsiveness to glucose stimulation was comparable to that of control islets (Figure 1B). As shown in Figure 1C, the presence of metformin led to a significant decline of cytokine-induced caspase 3/7 activity, a marker of cell apoptosis. These results show that metformin could counteract part of the deleterious actions of proinflammatory cytokines on human islet cells.

![Figure 1](image_url)

Figure 1. Effect of metformin on cytokine-induced $\beta$-cell damage. (A) Insulin content, reduced after cytokine-treatment, was marginally affected by metformin. (B) Insulin stimulation index was reduced after 48 h treatment with cytokines (Cyt) and tended to return to the control values (Ctrl) in islets treated with metformin (Cyt + Met). (C) Cytokines induced a significant activation of caspase 3/7, while metformin significantly reduced this activation. One to three replicates from three to four independent islet preparations were studied. The different groups were compared with One-way ANOVA followed by the Tukey correction. **** $p < 0.0001$, ** $p < 0.01$, * $p < 0.05$.

3.2. Identification of Islet Proteins Using Multidimensional Shotgun Proteomics

Isotope free shotgun proteomics analysis, performed after 1D-PAGE separation, was carried out in control and treated islets, to assess and identify differentially expressed proteins in cytokine- and cytokine plus metformin-exposed vs. control samples. A global view of the experimental workflow is shown in Figure 2A. In 1D gels, the 16 extracted bands were highlighted and paired to the corresponding average mass of the proteins identified in each band (Figure 2B). By merge-processing the 16 bands of each gel lane, altogether 3115 proteins were identified (Table S1): 1857 in subject 1; 2471 in subject 2; and 2585 in subject 3. The proteins identified across all samples were 1525 and those present in at least two preparations were 2271. Figure 2C shows a Venn diagram of the three series.
We then assessed the proteins significantly affected by the addition of cytokines and cytokines plus metformin. Taking into consideration the proteins present in at least two preparations, we found 244 proteins significantly affected by cytokines (145 up- and 99 down-regulated), of which 32 were exclusively detected in the cytokine-treated islets (Table S2). The addition of metformin to cytokine treatment significantly altered the expression of 231 proteins (128 up- and 103 downregulated), compared to control samples (Table S3). Of these, 19 were only detected in the cytokine-treated islets.

As shown in Figure 3A, 212 differentially expressed proteins were found in common between the two different comparisons (Table 1), mostly regulated in the same direction (98 up–up and 88 down–down, Figure 3B and Table S4). Interestingly, 26 proteins showed an inverse regulation compared to control islets (Table S4). Of these, 11 were downregulated by cytokine treatment and upregulated after the addition of metformin (Figure 3B). Most proteins were involved in vesicle motility (transgelin, Ras-related protein Rab-14), defense against oxidative stress (peroxiredoxins, PRDX2 and PRDX5) and metabolism (flavin reductase, mitochondrial ATP synthase subunit O). Among the 15 proteins upregulated by cytokines and downregulated by metformin (Figure 3B), we detected proteins involved in protein synthesis (40S ribosomal proteins S3, S6, S9, eukaryotic translation initiation factor 4E), glycolysis, or glycolysis regulation (triosephosphate isomerase, pyruvate kinase); protein folding and secretion (peptidyl-prolyl cis-trans isomerase FKBP2, protein disulfide-isomerase); and cytoskeletal proteins or proteins interacting with the cytoskeleton (myosin light polypeptide 6, Ras-related protein Ral-A, coactosin-like protein).
Figure 3. Comparison of differentially expressed proteins in cytokines vs. control and cytokines plus metformin vs. control. (A) Venn diagram showing the number of differentially expressed proteins in common between Cyt vs. Ctrl and Cyt + Met vs. Ctrl. (B) Mosaic plot showing the directionality of the significantly affected proteins in common between the two different comparisons.

Table 1. List of proteins significantly modulated by both cytokines and cytokines plus metformin vs. the respective control samples.

| ID     | Protein Name                                | Gene            | Cyt/Ctrl | Cyt + Met/Ctrl |
|--------|---------------------------------------------|-----------------|----------|----------------|
|        | Apoptosis                                   |                 |          |                |
| Q95140 | MITOFUSIN-2 (PTHR10465:SF1)                 | MFN2            | 0.01     | 0.01           |
| Q9H1Y0 | AUTOPHAGY PROTEIN 5 (PTHR13040:SF2)         | ATG5            | 0.01     | 0.01           |
| Q9NR28 | DIABLO HOMOLOG, MITOCHONDRIAL              | DIABLO          | 0.23     | 0.95           |
| Q13813 | SPECTRIN ALPHA CHAIN, NON-ERYTHROCYTIC 1 (PTHR11915:SF427) | SPTAN1          | 0.54     | 0.15           |
| Q13501 | SEQUESTOSOME-1 (PTHR15090:SF0)              | SQSTM1          | 2.06     | 1.8            |
|        | Cytoskeleton, vesicle motility/intracellular transport/vesicle release |                 |          |                |
| P13637 | SODIUM/POTASSIUM-TRANSPORTING ATPASE SUBUNIT ALPHA-3 (PTHR43294:SF15) | ATP1A3          | 0.01     | 0.01           |
| Q5D862 | FILAGGRIN-2 (PTHR22571:SF24)                | FLG2            | 0.01     | 0.01           |
| Q6KB66 | KERATIN, TYPE II CYTOSKELETAL 80           | KRT80           | 0.01     | 0.01           |
| Q8YZ3  | HORNERIN (PTHR22571:SF25)                   | HRNR            | 0.01     | 0.27           |
| Q8IV36 | PROTEIN HID1 (PTHR21575:SF12)              | HID1            | 0.01     | 0.01           |
| Q8N1N4 | KERATIN, TYPE II CYTOSKELETAL 78          | KRT78           | 0.01     | 0.01           |
|        | Cytoskeleton, vesicle motility/intracellular transport/vesicle release |                 |          |                |
| Q99442 | TRANSLLOCATION PROTEIN SEC62 (PTHR12443:SF9) | SEC62           | 0.01     | 0.01           |
| Q01995 | TRANSGELIN (PTHR18959:SF40)                 | TAGLN           | 0.09     | 1.11           |
| P61204 | ADP-RIBOSYLACTION FACTOR 3 (PTHR11711:SF316) | ARF3            | 0.1      | 0.98           |
| Q06141 | REGENERATING ISLET-DERIVED PROTEIN 3-ALPHA (PTHR22803:SF123) | REG3A           | 0.18     | 0.12           |
| Q94875 | SORBIN AND SH3 DOMAIN-CONTAINING PROTEIN 2 (PTHR14167:SF56) | SORBS2          | 0.19     | 0.2            |
| Q9BVK6 | DOMAIN-CONTAINING PROTEIN 9 (PTHR22811:SF37) | TMED9           | 0.2      | 0.85           |
| P08670 | VIMENTIN (PTHR45652:SF5)                    | VIM             | 0.32     | 0.32           |
| P61106 | RAS-RELATED PROTEIN RAB-14 (PTHR24073:SF185) | RAB14           | 0.35     | 1.59           |
| Q14019 | COACTOSIN-LIKE PROTEIN (PTHR10829:SF29)     | COTL1           | 1.14     | 0.4            |
| ID       | Protein Name                                      | Gene                      | Cyt/Ctrl | Cyt + Met/Ctrl |
|----------|---------------------------------------------------|---------------------------|----------|----------------|
| Q969Q5  | RAS-RELATED PROTEIN RAB-24 (PTHR24073:SF471)      | RAB24                     | 1.89     | 2.53           |
| P07737  | PROFILIN-1 (PTHR11936:SF14)                       |PFN1                       | 4.98     | 17.49          |
| Q9BQE5  | APOLIPOPROTEIN L2 (PTHR14096:SF27)                | APOL2                     | 6.47     | 5.93           |
|         | actin and actin related protein or actin-binding protein |              |          |                |
| Q9BYX7  | BETA-ACTIN-LIKE PROTEIN 3-RELATED (PTHR11937:SF278) | POTEKP                    | 0.01     | 0.01           |
| P68032  | ACTIN, ALPHA CARDIAC MUSCLE 1 (PTHR11937:SF176)   | ACTC1                     | 0.32     | 0.09           |
| P66660  | MYOSIN LIGHT POLYPEPTIDE 6 (PTHR23048:SF7)        | MYL6                      | 1.69     | 0.43           |
| P63261  | ACTIN, CYTOPLASMIC 2 (PTHR11937:SF414)            | ACTG1                     | 2        | 2.15           |
| O43795  | UNCONVENTIONAL MYOSIN-IB (PTHR13140:SF277)        | MYO1B                     | 2.98     | 3              |
| P02751  | FIBRONECTIN (PTHR19143:SF267)                     | FN1                       | 0.01     | 0.01           |
| O15240  | NEUROSECRETORY PROTEIN VGF (PTHR15159:SF2)        | VGF                       | 1.76     | 1.94           |
| Q9NRH3  | TUBULIN GAMMA-2 CHAIN (PTHR11588:SF79)            | TUBG2                     | 0.01     | 0.01           |
| Q71U36  | TUBULIN ALPHA-1A CHAIN (PTHR11588:SF133)          | TUBA1A                    | 1.27     |                |
|         | small GTPase                                      |                           |          |                |
| P62826  | GTP-BINDING NUCLEAR PROTEIN RAN (PTHR24071:SF23)  | RAN                       | 0.39     | 1.48           |
| P11233  | RAS-RELATED PROTEIN RAL-A (PTHR24070:SF174)       | RALA                      | 1.98     | 0.62           |
| P11234  | RAS-RELATED PROTEIN RAL-B (PTHR24070:SF199)       | RALB                      | 5.02     | 2.86           |
|         | Defense repair/immune response/Signaling in Immune system |              |          |                |
| P05451  | LITHOSTATHINE-1-ALPHA (PTHR22803:SF105)           | REG1A                     | 0.42     | 0.27           |
| P10599  | THIOREDOXIN (PTHR10438:SF18)                      | TXN                       | 1.79     | 2.26           |
| Q6323   | PROTEASOME ACTIVATOR COMPLEX SUBUNIT 1 (PTHR10660:SF5) | PSME1                    | 1.99     | 1.78           |
| Q9Y6N5  | SULFIDE:QUINONE OXIDOREDUCTASE, MITOCONDRIAL (PTHR10632:SF2) | SQOR                     | 2.1      | 3.46           |
| O15533  | TAPASIN (PTHR23411:SF22)                           | TAPBP                     | 3.97     | 6.54           |
| P13164  | INTERFERON-INDUCED TRANSMEMBRANE PROTEIN 1 (PTHR13999:SF6) | IFITM1                    | 4.8      | 4.98           |
| P05362  | INTERCELLULAR ADHESION MOLECULE 1 (PTHR13771:SF9)  | ICAM1                     | 24.83    | 23.94          |
| P00751  | COMPLEMENT FACTOR B (PTHR46393:SF1)                | CFB                       | 32.89    | 29.22          |
| P14174  | MACROPHAGE MIGRATION INHIBITORY FACTOR (PTHR11954:SF6) | MIF                      | 34.9     | 51.02          |
| P05161  | UBIQUITIN-LIKE PROTEIN ISG15 (PTHR10666:SF267)     | ISG15                     | 45.7     | 52.83          |
| Q10589  | BONE MARROW STROMAL ANTIGEN 2 (PTHR15190:SF1)      | BST2                      | 56.28    | 55.56          |
| Q14879  | INTERFERON-INDUCED PROTEIN WITH TETRATRICPEPTIDE REPEATS 3 (PTHR10271:SF3) | IFIT3                     | 69.33    | 64.83          |
| P09913  | INTERFERON-INDUCED PROTEIN WITH TETRATRICPEPTIDE REPEATS 2 (PTHR10271:SF4) | IFIT2                     | 100      | 100            |
| ID     | Protein Name                                                                 | Gene               | Cyt/Ctrl | Cyt + Met/Ctrl |
|--------|------------------------------------------------------------------------------|--------------------|----------|----------------|
| Q96AZ6 | INTERFERON-STIMULATED GENE 20 KDA PROTEIN (PTHR12801:SF59)                  | ISG20              | 100      | 96.33          |
|        | peroxidase                                                                   |                    |          |                |
| P30048 | THIREDOXIN-DEPENDENT PEROXIDE REDUCTASE, MITOCHONDRIAL (PTHR42801:SF4)      | PRDX3              | 0.18     | 0.95           |
| P32119 | PEROXIREDOXIN-2 (PTHR10681:SF161)                                           | PRDX2              | 0.34     | 1.02           |
| P30044 | PEROXIREDOXIN-5, MITOCHONDRIAN (PTHR10430:SF16)                             | PRDX5              | 0.46     | 1.8            |
| P30041 | PEROXIREDOXIN-6 (PTHR43503:SF11)                                            | PRDX6              | 0.79     | 0.14           |
| Q06830 | PEROXIREDOXIN-1 (PTHR10681:SF111)                                           | PRDX1              | 2.2      | 1.86           |
|        | oxidase/oxidoreductase                                                        |                    |          |                |
| O14618 | COPPER CHAPERONE FOR SUPEROXIDE DISMUTASE (PTHR10003:SF88)                 | CCS                | 0.23     | 0.01           |
| Q9NRD8 | DUAL OXIDASE 2 (PTHR11972:SF67)                                             | DUOX2              | 5.2      | 3.26           |
|        | SUPEROXIDE DISMUTASE [MN], MITOCHONDRIAN (PTHR11404:SF6)                    | SOD2               | 6.39     | 5.12           |
| P04179 | CERULOPLASMIN (PTHR11709:SF226)                                             | CP                 | 21.87    | 21.33          |
| P00450 | GAMMA-INTERFERON-INDUCIBLE LYSOSOMAL THIOL REDUCTASE (PTHR13234:SF8)        | IFI30              | 38.02    | 6.53           |
|        | heterotrimeric G-protein                                                      |                    |          |                |
| P32456 | GUANYLATE-BINDING PROTEIN 2 (PTHR10751:SF112)                               | GBP2               | 64.5     | 69             |
| Q96PP8 | GUANYLATE-BINDING PROTEIN 5 (PTHR10751:SF40)                                | GBP5               | 69.41    | 55.56          |
| P32455 | GUANYLATE-BINDING PROTEIN 1 (PTHR10751:SF96)                                | GBP1               | 72       | 72.8           |
| Q96PP9 | GUANYLATE-BINDING PROTEIN 4 (PTHR10751:SF17)                                | GBP4               | 100      | 100            |
|        | chemokine                                                                    |                    |          |                |
| P09341 | GROWTH-REGULATED ALPHA PROTEIN (PTHR10179:SF69)                             | CXCL1              | 11.21    | 21.38          |
| P78556 | C-C MOTIF CHEMOKINE 20 (PTHR12015:SF108)                                    | CCL20              | 57.22    | 55.56          |
| P19875 | C-X-C MOTIF CHEMOKINE 2 (PTHR10179:SF80)                                   | CXCL2              | 58.95    | 70             |
| P02778 | C-X-C MOTIF CHEMOKINE 10 (PTHR10179:SF47)                                   | CXCL10             | 100      | 100            |
|        | membrane traffic protein                                                     |                    |          |                |
| Q03169 | TUMOR NECROSIS FACTOR ALPHA-INDUCED PROTEIN 2 (PTHR21292:SF4)               | TNFAIP2            | 4.78     | 6.27           |
| P20591 | INTERFERON-INDUCED GTP-BINDING PROTEIN MX1 (PTHR11566:SF51)                 | MX1                | 39.72    | 43.43          |
|        | ATP-binding cassette (ABC) transporter                                       |                    |          |                |
| Q03518 | ANTIGEN PEPTIDE TRANSPORTER 1 (PTHR24221:SF249)                             | TAP1               | 10.59    | 9.41           |
| Q03519 | ANTIGEN PEPTIDE TRANSPORTER 2 (PTHR24221:SF237)                             | TAP2               | 13.9     | 11.72          |
|        | Cytokine and Interferon Signaling in Immune system                           |                    |          |                |
| P10145 | INTERLEUKIN-8 (PTHR10179:SF42)                                               | CXCL8              | 17.68    | 19.64          |
|        | major histocompatibility complex protein                                      |                    |          |                |
| P04439 | HLA CLASS I HISTOCOMPATIBILITY ANTIGEN, A ALPHA CHAIN (HUMAN LEUKOCYTE     | HLA-A              | 0        | 100            |
|        | ANTIGEN A) (HLA-A)                                                           |                    |          |                |
| P61769 | BETA-2-MICROGLOBULIN (PTHR19944:SF62)                                        | B2M                | 2.74     | 1.68           |
| P01911 | HLA CLASS II HISTOCOMPATIBILITY ANTIGEN, DRB1-15 BETA CHAIN (PTHR19944:SF99)| HLA-DRB1           | 14.79    | 15.21          |
| ID      | Protein Name                                                                 | Gene              | Cyt/Ctrl | Cyt + Met/Ctrl |
|---------|-------------------------------------------------------------------------------|-------------------|----------|---------------|
| P10321  | HLA CLASS I HISTOCOMPATIBILITY ANTIGEN, C ALPHA CHAIN (HLA-C) (HLA-CW)        | HLA-C             | 37.18    | 11.22         |
| P01903  | HLA CLASS II HISTOCOMPATIBILITY ANTIGEN, DR ALPHA CHAIN (PTHR19944:SF63)      | HLA-DRA           | 51.82    | 51.11         |
| P01889  | HLA CLASS I HISTOCOMPATIBILITY ANTIGEN, B ALPHA CHAIN                         | HLA-B             | 100      | 0             |
|         | **Metabolism**                                                                |                   |          |               |
| P09601  | HEME OXYGENASE 1 (PTHR10720:SF1)                                              | HMOXI             | 0.01     | 0.01          |
| P34059  | N-ACETYLGALACTOSAMINE-6-SULFATASE (PTHR42693:SF33)                            | GALNS             | 0.01     | 0.01          |
| P16233  | PANCREATIC TRIACYLGLYCEROL LIPASE (PTHR11610:SF115)                           | PNLIP             | 0.21     | 0.38          |
| P04054  | PHOSPHOLIPASE A2 (PTHR11716:SF94)                                            | PLA2G1B           | 0.33     | 0.01          |
| Q8NCW5  | NAD(P)H-HYDRATE EPIMERASE (PTHR13232:SF11)                                    | NAXE              | 0.41     | 0.12          |
| Q9H2U2  | INORGANIC PYROPHOSPHATASE 2, MITOCHONDRIAL (PTHR10286:SF49)                   | PPA2              | 0.69     | 0.11          |
| P47985  | CYTOCHROME B-C1 COMPLEX SUBUNIT RIESKE, MITOCHONDRIAL-RELATED (PTHR10134:SF20) | UQCRFS1           | 1.55     | 2.83          |
| P15954  | CYTOCHROME C OXIDASE SUBUNIT 7C, MITOCHONDRIAL (PTHR13313:SF1)                | COX7C             | 2.42     | 3.84          |
| P19971  | THYMIDINE PHOSPHORYLASE (PTHR10515:SF0)                                       | TYMP              | 5.68     | 6.01          |
|         | **ATP synthase**                                                              |                   |          |               |
| P06576  | ATP SYNTHASE SUBUNIT BETA, MITOCHONDRIAL (PTHR15184:SF58)                    | ATPSF1B           | 0.28     | 0.52          |
| P48047  | ATP SYNTHASE SUBUNIT O, MITOCHONDRIAL (PTHR191910:SF1)                       | ATPSPO            | 0.65     | 1.11          |
|         | **kinase**                                                                    |                   |          |               |
| P07205  | PHOSPHOGLYCERATE KINASE 2 (PTHR11406:SF10)                                   | PGK2              | 0.01     | 0.01          |
| Q6ZUJ8  | PHOSPHOINOSITIDE 3-KINASE ADAPTER PROTEIN 1 (PTHR16267:SF12)                  | PIK3AP1           | 2.89     | 2.79          |
| P14618  | PYRUVATE KINASE PKM (PTHR11817:SF15)                                          | PKM               | 6.41     | 0.47          |
| P32189  | GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE (PTHR10836:SF11)                    | GAPDH             | 0.33     | 0.86          |
| Q8NBQ5  | ESTRADIOL 17-BETA-DEHYDROGENASE 11 (PTHR24322:SF489)                          | HSD17B11          | 0.5      | 0.04          |
| P15559  | NAD(P)H DEHYDROGENASE [QUINONE] 1 (PTHR10204:SF1)                            | NQO1              | 0.31     | 0.25          |
| P04406  | GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE (PTHR10836:SF11)                    | GAPDH             | 0.33     | 0.86          |
| Q8NBQ5  | ESTRADIOL 17-BETA-DEHYDROGENASE 11 (PTHR24322:SF489)                          | HSD17B11          | 0.5      | 0.04          |
| P40926  | MALATE DEHYDROGENASE, MITOCHONDRIAL (PTHR11540:SF16)                         | MDH2              | 0.51     | 0.15          |
| P47989  | XANTHINE DEHYDROGENASE/OXIDASE (PTHR11908:SF80)                               | XDH               | 60.25    | 62.5          |
| P14902  | INDOLEAMINE 2,3-DIOXGENASE 1 (PTHR28657:SF2)                                  | IDO1              | 100      | 100           |
|         | **isomerase**                                                                 |                   |          |               |
| Q13907  | ISOPENTENYL-DIPHOSPHATE DELTA-ISOMERASE 1 (PTHR10885:SF5)                     | IDI1              | 0.5      | 0.01          |
| P60174  | TRIOSEPHOSPHATE ISOMERASE (PTHR21139:SF24)                                    | TPI1              | 1.29     | 0.18          |
|         | **transferase**                                                                |                   |          |               |
| P51580  | THIOPURINE S-METHYLTRANSFERASE (PTHR10259:SF11)                               | TPMT              | 0.01     | 0.01          |
| ID       | Protein Name                                      | Gene               | Cyt/Ctrl | Cyt + Met/Ctrl |
|----------|--------------------------------------------------|--------------------|----------|----------------|
| P08263   | GLUTATHIONE S-TRANSFERASE A1 (PTHR11571:SF157)  | GSTA1              | 0.29     | 0.62           |
| P09210   | GLUTATHIONE S-TRANSFERASE A2 (PTHR11571:SF243) | GSTA2              | 0.31     | 0.62           |
| Q99735   | MICROSOMAL GLUTATHIONE S-TRANSFERASE 2 (PTHR10250:SF13) | MGST2             | 0.36     | 0.21           |
| P24752   | ACETYL-COA ACETYLTRANSFERASE, MITOCHONDRIAL (PTHR18919:SF156) | ACAT1             | 0.43     | 0.86           |
| Q14749   | GLYCINE N-METHYLTRANSFERASE (PTHR16458:SF2)     | GNMT               | 0.44     | 0.01           |
| P40261   | NICOTINAMIDE N-METHYLTRANSFERASE (PTHR10867:SF37) | NNMT              | 1.99     | 2.17           |
| P43490   | PHOSPHORIBOSYLTRANSFERASE (PTHR43816:SF1)      | NAMPT              | 2.33     | 2.55           |
| P21980   | GAMMA-Glutamyltransferase 2 (PTHR11590:SF6)    | TGM2               | 3.39     | 2.62           |
| P30043   | FLAVIN REDUCTASE (NADPH) (PTHR43355:SF2)       | BLVRB              | 0.36     | 1.45           |
| P52895   | ALDO-KETO REDUCTASE FAMILY 1 MEMBER C1-RELATED (PTHR1732:SF153) | AKR1C2           | 2.87     | 2.21           |
| Q32P28   | PROLYL 3-HYDROXYLASE 1 (PTHR14049:SF5)         | P3H1               | 0.01     | 0.01           |
| Q6bJ6    | LYSYL OXIDASE HOMOLOG 4 (PTHR45817:SF5)        | LOXL4              | 0.01     | 0.01           |
| P61278   | SOMATOSTATIN (PTHR10558:SF2)                   | SST                | 0.26     | 0.01           |
| P16870   | CARBOXYPEPTIDASE E (PTHR11332:SF62)            | CPE                | 0.59     | 0.42           |
| P19009   | CLUSTERIN (PTHR10970:SF1)                      | CLU                | 0.63     | 0.21           |
| P07237   | PROTEIN DISULFIDE-ISOMERASE (PTHR18929:SF101) | P4HB               | 1.2      | 0.42           |
| P01275   | GLUCAGON (PTHR11418:SF0)                       | GCG                | 1.9      | 0.73           |
| P26885   | PEPTIDYL-PROLYL CIS-TRANS ISOMERASE            | FKB2               | 1.98     | 0.67           |
| P28062   | PROTEASOME SUBUNIT ETA TYPE-8 (PTHR11599:SF53) | PSMB8              | 2.06     | 2.47           |
| P11021   | ENDOPLASMIC RETICULUM CHAPERONE BIP (PTHR19375:SF380) | HSPA5             | 2.18     | 1.39           |
| Q00653   | NUCLEAR FACTOR NF-KAPPA-B P100 SUBUNIT (DNA-BINDING FACTOR KBF2) (H2TF1)] | NFKB2             | 3.28     | 2.9            |
| Q9UL46   | PROTEASOME ACTIVATOR COMPLEX SUBUNIT 2 (PTHR10660:SF6) | PSME2             | 3.7      | 3.51           |
| P10645   | CHROMOGRANIN- A (CgA)                          | CHGA               | 3.86     | 5.24           |
| P29590   | PROTEIN PML (PTHR25462:SF241)                  | PML                | 4.64     | 4.39           |
| P28065   | PROTEASOME SUBUNIT ETA TYPE-9 (PTHR11599:SF50) | PSMB9              | 6.02     | 5.32           |
| P40306   | PROTEASOME SUBUNIT ETA TYPE-10 (PTHR11599:SF41) | PSMB10             | 10.69    | 13.67          |
| P01833   | POLYMERIC IMMUNOGLOBULIN RECEPTOR (PTHR11860:SF82) | PIGR              | 19       | 15.97          |
| O15205   | UBIQUITIN D (PTHR47731:SF1)                     | UBD                | 62.5     | 55.63          |
| Q99797   | MITOCHONDRIAL INTERMEDIATE PEPTIDASE (PTHR11804:SF5) | MIPEP             | 0.01     | 0.01           |
| Q6P179   | ENDOPLASMIC RETICULUM AMINOPEPTIDASE 2 (PTHR11533:SF239) | ERAP2            | 3.56     | 35.71          |
| ID      | Protein Name                                      | Gene                      | Cyt/Ctrl | Cyt + Met/Ctrl |
|---------|--------------------------------------------------|---------------------------|----------|----------------|
| P28538  | CYTOSOL AMINOPEPTIDASE (PTHR11963:SF39)          | LAP3                      | 6.61     | 7.38           |
| P09936  | UBIQUITIN CARBOXYL-TERMINAL HYDROLASE ISOZYME L1 (PTHR10589:SF19) | UCHL1                    | 0.62     | 0.22           |
| P25774  | CATHEPSIN S (PTHR12411:SF25)                     | CTSS                      | 4.99     | 5.02           |
| Q9ULP0  | PROTEIN NDRG4 (PTHR11034:SF21)                   | NDRG4                     | 0.01     | 0.01           |
| P07478  | TRYPsin-2 (PTHR24264:SF53)                       | PRSS2                     | 0.36     | 0.31           |
| P07477  | TRYPsin-1 (PTHR24264:SF59)                       | PRSS1                     | 0.44     | 0.37           |
| Q6GP11  | CHYMOTRYPSINOGEN B2 (PTHR24250:SF53)             | CTRB2                     | 0.46     | 0.46           |
| P52815  | 39S RIBOSOMAL PROTEIN L12, MITOCHONDRIAL (PTHR45987:SF4) | MRPL12                   | 0.01     | 0.01           |
| P05387  | 60S ACIDIC RIBOSOMAL PROTEIN P2 (PTHR21141:SF5) | RPLP2                     | 0.35     | 0.32           |
| Q96A35  | 39S RIBOSOMAL PROTEIN L24, MITOCHONDRIAL (PTHR12903:SF0) | MRPL24                   | 0.4      | 0.01           |
| Q9H2W6  | 39S RIBOSOMAL PROTEIN L46, MITOCHONDRIAL (PTHR13124:SF12) | MRPL46                   | 0.48     | 0.02           |
| Q9H9J2  | 39S RIBOSOMAL PROTEIN L44, MITOCHONDRIAL (PTHR11207:SF5) | MRPL44                   | 0.59     | 0.14           |
| P62587  | 40S RIBOSOMAL PROTEIN S28 (PTHR10769:SF3)        | RPS28                     | 0.61     | 0.45           |
| P62753  | 40S RIBOSOMAL PROTEIN S6 (PTHR11502:SF16)        | RPS6                      | 1.42     | 0.92           |
| P23396  | 40S RIBOSOMAL PROTEIN S3 (PTHR11760:SF36)        | RPS3                      | 1.57     | 0.32           |
| P60866  | 40S RIBOSOMAL PROTEIN S20 (PTHR11700:SF8)        | RPS20                     | 1.87     | 1.31           |
| P46781  | 40S RIBOSOMAL PROTEIN S9 (PTHR11831:SF23)        | RPS9                      | 2.19     | 0.96           |
| Q99816  | TUMOR SUSCEPTIBILITY GENE 101 PROTEIN (PTHR23306:SF17) | TSG101                   | 0.35     | 1.38           |
| O14933  | UBIQUITIN/ISG15-CONJUGATING ENZYME E2 L6 (PTHR24068:SF43) | UBE2L6                   | 11.67    | 10.77          |
| Q13123  | PROTEIN RED (PTHR12765:SF7)                      | IK                        | 0.01     | 0.01           |
| Q9NR30  | NUCLEOLAR RNA HELICASE 2 (PTHR47958:SF109)       | DDX21                     | 0.01     | 0.01           |
| Q96EP5  | DAZ-ASSOCIATED PROTEIN 1 (PTHR48027:SF12)        | DAZAP1                    | 0.2      | 0.49           |
| Q9H583  | HEAT REPEAT-CONTAINING PROTEIN 1 (PTHR3457:SF1)  | HEATR1                    | 2.24     | 7.58           |
| P23381  | TRYPTOPHAN-TRNA LIGASE, CYTOPLASMIC (PTHR10055:SF1) | WARS1                    | 18.27    | 21.83          |
| P55769  | NHP2-LIKE PROTEIN 1 (PTHR23105:SF38)             | SNU13                     | 23.22    | 51.02          |
| Q9UI30  | MULTIFUNCTIONAL METHYLTTRANSFERASE SUBUNIT TRM112-LIKE PROTEIN (PTHR21277:SF2) | TRMT112                  | 51.09    | 50.85          |
| P62304  | SMALL NUCLEAR RIBONUCLEOPROTEIN E (PTHR11193:SF9) | SNRPE                     | 51.39    | 50.61          |
| Q9Y6K5  | 2'-5'-OLIGOADENYLATE SYNTHASE 3 (PTHR11258:SF4)  | OAS3                      | 52.63    | 52.68          |
| Q53EL6  | PROGRAMMED CELL DEATH PROTEIN 4 (PTHR12626:SF3)  | PDCD4                     | 0.32     | 0.32           |
| P06730  | EUKARYOTIC TRANSLATION INITIATION FACTOR 4E (PTHR1960:SF14) | EIF4E                    | 1.67     | 0.68           |
| P68104  | ELONGATION FACTOR 1-ALPHA 1-RELATED (PTHR23115:SF22) | EEF1A1                   | 2.11     | 2.37           |
| P84243  | HISTONE H3.3-RELATED (PTHR11426:SF228)           | H3-3A                     | 0.79     |                |
| P62805  | HISTONE H4 (PTHR10484:SF163)                     | H4-16                     | 2        | 2.45           |
Table 1. Cont.

| ID       | Protein Name                                         | Gene                          | Cyt/Ctrl | Cyt + Met/Ctrl |
|----------|------------------------------------------------------|-------------------------------|----------|---------------|
| Q1677    | HISTONE H2A TYPE 2-C (PTHR23430:SF130)              | H2AC20                        | 2.75     | 1.25          |
| P0C65    | HISTONE H2A.Z (PTHR23430:SF47)                       | H2AZ1                         | 34.01    | 51.25         |
| Q7L7L0   | HISTONE H2A TYPE 3 (PTHR23430:SF220) DNA binding    | H2AW                          | 51.25    | 50.64         |
| P43246   | DNA MISMATCH REPAIR PROTEIN MSH2 (PTHR11361:SF35)   | MSH2                          | 0.01     | 0.01          |
| P12004   | PROLIFERATING CELL NUCLEAR ANTIGEN (PTHR11352:SF5)  | PCNA                          | 0.81     | 0.25          |
| P42224   | SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION 1-ALPHA/BETA (PTHR11801:SF18) | STAT1                           | 9.25     | 9.63          |
| P13521   | SECRETOGRAFIN-2 (PTHR15119:SF0)                      | SCG2                          | 0.45     | 0.21          |
| Q12860   | CONTACTIN-1 (PTHR41470:SF10)                         | CNTN1                         | 0.01     | 0.01          |
| Q14966   | ZINC FINGER PROTEIN 638 (PTHR15592:SF1)              | ZNF638                        | 0.01     | 0.01          |
| Q5BJF2   | SIGMA INTRACELLULAR RECEPTOR 2 (PTHR12104:SF1)       | TMEM97                        | 0.01     | 0.01          |
| Q96Z3    | MITOCHONDRIAL AMIDOXIME REDUCING COMPONENT 2 (PTHR14237:SF27) | MTAR2C                          | 0.01     | 0.01          |
| Q9BZH6   | WD REPEAT-CONTAINING PROTEIN 11 (PTHR14593:SF5)      | WDR11                         | 0.01     | 0.01          |
| Q8WY91   | THAP DOMAIN-CONTAINING PROTEIN 4 (PTHR15584:SF4)     | THAP4                         | 0.17     | 50.62         |
| Q9YW2W1  | THYROID HORMONE RECEPTOR-ASSOCIATED PROTEIN 3 (PTHR15268:SF16) | THRAP3                          | 0.41     | 0.22          |
| P05408   | NEUROENDOCRINE PROTEIN 7B2 (PTHR12738:SF0)           | SCG5                          | 0.44     | 0.01          |
| P48059   | ANTIGEN-LIKE-CONTAINING DOMAIN PROTEIN 1 (PTHR24210:SF11) | LIMS1                          | 0.45     | 0.41          |
| O14997   | CALMEGIN (PTHR11073:SF7)                             | CLGN                          | 0.49     | 0.17          |
| P05600   | SECRETOGRAFIN-1 (PTHR10583:SF4)                      | CHGB                          | 0.52     | 0.37          |
| P02766   | TRANSTHYRETIN (PTHR10395:SF12)                       | TTR                          | 0.61     | 0.26          |
| Q9UHC3   | PRENYLCYSTEINE OXIDASE 1 (PTHR15944:SF3)             | PCYOX1                        | 0.85     | 0.21          |
| Q75323   | PROTEIN NIPSAP HOMOLOG 2 (PTHR21017:SF14)            | NIPSAP2                       | 0.96     | 0.22          |
| Q96C19   | EF-HAND DOMAIN-CONTAINING PROTEIN D2 (PTHR13025:SF2) | EFHD2                         | 1.57     | 0.77          |
| Q12907   | VESICULAR INTEGRAL-MEMBRANE PROTEIN VIP36 (PTHR12223:SF36) | LMAN2                          | 2.14     | 2.75          |
| Q6UVJ0   | SPINDLE ASSEMBLY ABNORMAL PROTEIN 6 HOMOLOG (PTHR44281:SF1) | SASS6                          | 2.35     | 3.32          |
| Q9UKY7   | PROTEIN CDV3 HOMOLOG (PTHR16284:SF13)                | CDV3                          | 2.57     | 1.63          |
| Q9C002   | NORMAL MUCOSA OF ESOPHAGUS-SPECIFIC GENE 1 PROTEIN (PTHR14256:SF3) | NMES1                          | 2.61     | 3.12          |
| Q8N339   | METALLOTHIONEIN-1M (PTHR23299:SF50) NEUROBLAST      | MT1M                          | 3.11     | 2.49          |
| Q96666   | DIFFERENTIATION-ASSOCIATED PROTEIN AHNK (PTHR23348:SF41) | AHNK                          | 9.48     | 0.31          |
| Q63HN8   | E3 UBIQUITIN-PROTEIN LIGASE RNF213 (PTHR22605:SF16)   | RNF213                        | 10.35    | 8.25          |
| O95786   | ATP-DEPENDENT RNA HELICASE DDX58-RELATED (PTHR14074:SF16) | DDX58                          | 11.21    | 10.63         |
| P69905   | HEMOGLOBIN SUBUNIT ALPHA (PTHR11442:SF48)            | HBA1                          | 23.19    | 34.96         |
| Q13113   | PDZK1-INTERACTING PROTEIN 1 (PTHR15296:SF0)          | PDZK1IP1                      | 27.08    | 38.44         |
3.3. Gene Ontology, Analysis of Canonical Pathway, and Upstream Regulators

To investigate the biological meaning of the above-mentioned proteomics changes, the curated lists of differentially expressed proteins, namely cytokines vs. control and cytokines plus metformin vs. control conditions were submitted to gene ontology enrichment analysis (https://metascape.org/, accessed on 7 July 2022) [55]. The enrichment analysis results for the biological process and reactome curated pathways are shown in Figure 4. The most significantly enriched pathway includes 57 proteins annotated as “Cytokine Signaling in Immune system”, with a logP score Enrichment of −39.89. GO results for the biological processes annotation indicates that most proteins were related to the immune system response and response to cytokines. The above-described overlap of the proteins modulated by cytokines and cytokines plus metformin (n: 212) led to the similarity of the GO-enriched terms in the two conditions. Therefore, to better discriminate the effects of metformin in the presence of pro-inflammatory cytokines in human islets, ingenuity pathway analysis (IPA) was used. Table 2 shows the top 25 canonical pathways obtained from protein differentially expressed in cytokine-treated samples compared to the control. As expected, most of them resulted as associated with inflammatory processes. Those with the highest p-values were linked to the “Antigen Presentation Pathway”, “Phagosome Maturation”, “Acute Phase Response Signaling”, and “Interferon Signaling”. Other inflammation-related pathways such as the “IL-17 Signaling” and “Neuroinflammation Signaling”; and some stress-related pathways, such as the “NRF2-mediated Oxidative Stress Response”, “Mitochondrial Disfunction”; and “Type 1 Diabetes Mellitus Signaling” also resulted as significantly enriched. The upstream regulators resulting from IPA confirmed the activation of several transcription factors, such as STAT1, IRF1, RELA, and IRF7, with high positive values of z-score. In addition, a significant inhibition of MAPK1 was shown. The top 25 upstream regulators are listed in Table 3.

Table 1. Cont.

| ID     | Protein Name                                           | Gene     | Cyt/Ctrl | Cyt + Met/Ctrl |
|--------|--------------------------------------------------------|----------|----------|---------------|
| P58546 | MYOTROPHIN (PTHR24189:SF52) calcium-binding protein   | MTPN     | 67.35    | 50.64         |
| P0DP23 | CALMODULIN-1                                           | CALM1    | 0.43     | 0.43          |
| P09525 | ANNEXIN A4 (PTHR10502:SF28)                           | ANXA4    | 0.86     | 0.19          |
| P04083 | ANNEXIN A1 (PTHR10502:SF17) scaffold/adaptor protein | ANXA1    | 2.16     | 3.23          |
| Q9Y4E1 | WASH COMPLEX SUBUNIT 2A-RELATED (PTHR21669:SF1)        | WASHC2C  | 0.01     | 0.01          |
| P43487 | RAN-SPECIFIC GTPASE-ACTIVATING PROTEIN (PTHR23138:SF133) transfer/carryer protein | RANBP1 | 0.45 | 0.03 |
| P02753 | RETINOL-BINDING PROTEIN 4 (PTHR11873:SF2)              | RBP4     | 0.15     | 0.52          |
| O15127 | SECRETORY CARRIER-ASSOCIATED MEMBRANE PROTEIN 2 (PTHR10687:SF7) | SCAMP2 | 0.46 | 0.19 |
| P80188 | NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN (PTHR11430:SF13) protease inhibitor | LCN2     | 4.76     | 5.59          |
| P01011 | ALPHA-1-ANTICHYMOTRYPSIN (PTHR11461:SF145)             | SERPINA3 | 0.39     | 0.31          |
| P18065 | INSULIN-LIKE GROWTH FACTOR-BINDING PROTEIN 2 (PTHR11551:SF5) | IGFBP2 | 0.44 | 0.01 |
| P17936 | INSULIN-LIKE GROWTH FACTOR-BINDING PROTEIN 3 (PTHR11551:SF3) | IGFBP3 | 2.39 | 2.03 |
| P01024 | COMPLEMENT C3 (PTHR11412:SF81)                         | C3       | 7.03     | 7.25          |
| P05120 | PLASMINOGEN ACTIVATOR INHIBITOR 2 (PTHR11461:SF61)     | SERPINB2 | 8.1      | 7.19          |
| P48594 | SERPIN B4 (PTHR11461:SF186)                            | SERPINB4 | 100      | 106           |
Figure 4. Enrichment analysis results for biological process. (A) All statistically enriched terms were identified and used for filtering, after computing the accumulative hypergeometric p-values and enrichment factors. The remaining significant terms were then hierarchically clustered into a tree based on Kappa-statistical similarities among their gene memberships. Then a 0.3 kappa score was applied as a threshold to divide the tree into term clusters. The term with the best p-value within each cluster was selected as its representative term and displayed in a dendrogram. The heatmap cells are colored by their p-values. (B) A subset of representative terms from the full cluster were selected and converted into a network layout. Each term is represented by a circle node, where its size is proportional to the number of input genes fall into that term, and its color represent its cluster identity (i.e., nodes of the same color belong to the same cluster). Terms with a similarity score >0.3 are linked by an edge (the thickness of the edge represents the similarity score). The network was visualized with Cytoscape (v3.1.2) with “force-directed” layout and with edges bundled for clarity. One term from each cluster was selected to have its term description shown as a label. (C) The same enrichment network has its nodes colored by p-value, as shown in the legend. The darker the color, the more statistically significant the node is.
Table 2. Top 25 canonical pathways derived from the IPA analysis.

| Ingenuity Canonical Pathways                        | −log(p-Value) | z-Score | Molecules                                                                 |
|----------------------------------------------------|---------------|---------|---------------------------------------------------------------------------|
| Antigen Presentation Pathway                       | 11.2          |         | B2M, HLA-B, HLA-C, HLA-DRA, HLA-DRB1, PSMB8, PSMB9, TAP1, TAP2, TAPBP     |
| Phagosome Maturation                               | 10.2          |         | B2M, CTSS, HLA-B, HLA-C, HLA-DRA, HLA-DRB1, PRDX1, PRDX2, PRDX3, PRDX6, TAP1, TSG101, TUBA1A, TUBA4B, TUBG2 |
| NRF2-mediated Oxidative Stress Response            | 8.8           | 1.89    | ACTA1, ACTC1, ACTG1, GSTA1, GSTA2, HMOX1, MAP2K3, MGST2, NQO1, PRDX1, RALA, RALB, SOD2, SQSTM1, TXN |
| Protein Ubiquitination Pathway                     | 7.47          |         | B2M, HLA-B, HLA-C, HSP5, PSMB10, PSMB8, PSMB9, PSMD10, PSME1, PSME2, SASS6, TAP1, TAP2, UBD, UBE2L6, UCHL1 |
| Acute Phase Response Signaling                     | 7.25          | 2.111   | C3, CFB, CP, FN1, HMOX1, MAP2K3, NFKB2, RALA, RALB, RBP4, SERPINA3, SOD2, TTR |
| Interferon Signaling                               | 7.09          | 2.646   | IFIT1, IFITM1, ISG15, MX1, PSMB8, STAT1, TAP1                            |
| EIF2 Signaling                                     | 6.12          |         | ACTA1, ACTC1, EIF4E, HSPA5, RALA, RALB, RPLP2, RPS20, RPS28, RPS3, RPS6, RPS9, WARS1 |
| Crosstalk between Dendritic Cells and Natural Killer Cells and Diapedesis | 5.36          |         | ACTA1, ACTC1, ACTG1, HLA-B, HLA-C, HLA-DRA, HLA-DRB1, NFKB2             |
| Agranulocyte Adhesion and Diapedesis               | 5.19          |         | ACTA1, ACTC1, ACTG1, CCL20, CXCL1, CXCL10, CXCL2, CXCL8, FN1, ICAM1, MYL6 |
| Glycolysis I                                       | 5.18          | 0.447   | BPGM, GAPDH, PGK2, PKM, TPII                                            |
| Virus Entry via Endocytic Pathways                 | 4.76          |         | ACTA1, ACTC1, ACTG1, B2M, HLA-B, HLA-C, RALA, RALB                       |
| Neuroinflammation Signaling Pathway                | 4.74          | 3.051   | B2M, CXCL10, CXCL8, HLA-B, HLA-C, HLA-DRA, HLA-DRB1, HMOX1, ICAM1, NFKB2, PLA2G1B, SOD2, STAT1 |
| Type I Diabetes Mellitus Signaling Pathway         | 4.65          |         | CPE, HLA-B, HLA-C, HLA-DRA, HLA-DRB1, MAP2K3, NFKB2, STAT1              |
| Role of IL-17A in Arthritis                        | 4.63          |         | CCL20, CXCL1, CXCL2, CXCL8, MAP2K3, NFKB2                                |
| FAT10 Signaling Pathway                            | 4.49          |         | PSME1, PSME2, SQSTM1, UBD                                                |
| Regulation of eIF4 and p70S6K Signaling            | 4.36          | 1       | EIF4E, PPP2RB1, RALA, RALB, RPS20, RPS28, RPS3, RPS6, RPS9               |
| Activation of IRF by Cytosolic Pattern Recognition Receptors | 4.29          | 1.633   | DDX58, IFIH1, IFIT2, ISG15, NFKB2, STAT1                                 |
| Sirtuin Signaling Pathway                          | 4.21          | 0.333   | ATG5, ATP5F1B, BPGM, CXCL8, MT-ATP6, NAMPT, NFKB2, NQO1, SOD2, TUBA1A, TUBA4B, UQCRFS1 |
| Communication between Innate and Adaptive Immune Cells | 4.16          |         | B2M, CXCL10, CXCL8, HLA-B, HLA-C, HLA-DRA, HLA-DRB1                   |
| mTOR Signaling                                     | 4.10          | 0.816   | EIF4E, HMOX1, PPP2RB1, RALA, RALB, RPS20, RPS28, RPS3, RPS6, RPS9         |
| Remodeling of Epithelial Adherens Junctions        | 4.10          |         | ACTA1, ACTC1, ACTG1, MAPRE2, RALA, TUBA1A                                |
| Mitochondrial Dysfunction                          | 4.08          |         | ATP5F1B, ATP5F0, COX7C, MT-ATP6, PRDX3, PRDX5, SOD2, UQCRFS1, XDH      |
| Caveolar-mediated Endocytosis Signaling            | 3.93          |         | ACTA1, ACTC1, ACTG1, B2M, HLA-B, HLA-C                                 |
| IL-17A Signaling in Gastric Cells                  | 3.90          |         | CCL20, CXCL1, CXCL10, CXCL8                                            |
| Xenobiotic Metabolism General Signaling Pathway    | 3.87          | −0.707  | GSTA1, GSTA2, HMOX1, MAP2K3, MGST2, NQO1, RALA, RALB                   |
Table 3. Top 25 upstream regulators derived from IPA analysis of differentially expressed proteins obtained in the cytokines vs. control comparison.

| Upstream Regulator | Molecule Type | Predicted Activation State | Activation z-Score | p-Value of Overlap | Target Molecules in Dataset |
|--------------------|---------------|-----------------------------|-------------------|-------------------|-----------------------------|
| IFNG                | cytokine      | Activated                   | 6.4               | $1.27 \times 10^{-27}$ | ATG5, B2M, BST2, C3, CCL20, CFB, CP, CTSS, CXCL1, CXCL10, CXCL2, CXCL8, DDX58, DIABLO, DUOX2, EEF1A1, ERAP2, FN1, GBP1, GBP2, GBP4, GBP5, HLA-B, HLA-C, HLA-DRA, HLA-DRB1, HMOX1, HSPA5, ICAM1, ID1, IDO1, IFI30, IFIH1, IFIT2, IFIT3, IFITM1, ISG15, ISG20, LCN2, MIF, MSH2, MX1, NAMPT, NDRG4, NFKB2, NQO1, OAS3, PIGR, PKM, PML, PRDX2, PSMB10, PSMB8, PSMB9, PSME1, PSME2, SG5, SOD2, SQSTM1, STAT1, TAP1, TAP2, TAPBP, TNAIP2, TFIIH, TYMP, UBD, UBE2L6, WARS1, ZNF638 |
| IRF1               | transcription regulator | Activated | 3.6               | $1.44 \times 10^{-26}$ | B2M, CCL20, CFB, CTSS, CXCL10, CXCL2, CXCL8, DDX58, GBP2, IDO1, IFIH1, IFIT2, IFIT3, IFITM1, ISG15, MX1, OAS3, PCNA, PIGR, PML, PSMB10, PSMB8, PSMB9, PSME1, PSME2, STAT1, TAP1, TAP2, TAPBP, UBD |
| STAT1              | transcription regulator | Activated | 5.4               | $3.13 \times 10^{-26}$ | B2M, BST2, C3, CCL20, CFB, CTSS, CXCL10, CXCL2, CXCL8, DUOX2, GBP1, GBP2, GBP5, ICAM1, IDO1, IFI30, IFIH1, IFIT2, IFIT3, IFITM1, ISG15, LCN2, MX1, OAS3, PSMB10, PSMB8, PSMB9, PSME1, PSME2, RNF213, SERPINA3, SERPINB4, STAT1, TAP1, TYMP, UBD, WARS1 |
| TNF                | cytokine      | Activated                   | 5.9               | $1.43 \times 10^{-24}$ | ACTA1, ANXA1, B2M, BPGM, BST2, C3, CCL20, CFB, CLU, COTL1, CP, CTSS, CXCL1, CXCL10, CXCL2, CXCL8, DDX58, EFHD2, FN1, GBP1, GBP2, GBP4, GSTA1, H1D1, HLA-B, HLA-DRA, HMOX1, HSPA5, ICAM1, IDO1, IFIH1, IFIT3, IFITM1, IGFBP2, IGFBP3, ISG15, LCN2, MAP2K3, MGST2, MIF, MSH2, MX1, MYL6, NAMPT, NFKB2, NNMT, NQO1, OAS3, P4HB, PCNA, PIGR, PKM, PML, PPP2R1B, PSMB10, PSMB8, PSMB9, PSME1, PSME2, PSME3, SERPINA3, SERPINB2, SOD2, SQSTM1, STAT1, TAC1N, TAP1, TAPBP, TGM2, TNAIP2, TXN, TYMP, UBD, VIM, XDH |
| OSM                | cytokine      | Activated                   | 4.3               | $1.33 \times 10^{-23}$ | ACTG1, AKR1C1, ANXA1, B2M, C2, CCL20, CXCL1, CXCL10, CXCL2, CXCL8, FN1, GBP1, GBP2, HLA-B, HLA-C, HMOX1, HSPA5, ICAM1, IGFBP3, ISG20, LCN2, MX1, NAMPT, PCNA, PDCDC4, PDZK1P1, PIGR, PRDX2, PSMB8, PSMB9, REG3A, SERPINA3, SERPINB4, SNRPE, STAT1, TAP1, TAP2, TAPBP, TNAIP2, TUBA1A, TYMP, UBE2L6, VIM, XDH |
| IFNA2              | cytokine      | Activated                   | 4.8               | $4.02 \times 10^{-23}$ | ACTG1, ANXA4, BST2, CPE, CXCL1, HLA-DRA, HMOX1, ID1, IFIH1, IFIT2, IFIT3, IFITM1, ISG15, ISG20, MTA5P6, MTM1, MX1, OAS3, PLA2G1B, PML, PSME1, STAT1, TAP1, TAP2, UBD, UBE2L6 |
| PML                | transcription regulator | Activated | 2.9               | $7.25 \times 10^{-23}$ | ACTG1, ANXA4, BST2, CPE, CXCL1, HLA-DRA, HMOX1, ID1, IFIH1, IFIT2, IFIT3, IFITM1, ISG15, ISG20, MX1, NQO1, OAS3, PML, PRDX1, PSMB8, PSMB9, SQSTM1, STAT1, TAP1, TAP2, TXN, VIM |
## Table 3. Cont.

| Upstream Regulator | Molecule Type | Predicted Activation State | Activation z-Score | p-Value of Overlap | Target Molecules in Dataset |
|--------------------|---------------|----------------------------|--------------------|-------------------|----------------------------|
| IL1B cytokine      | Activated     | 5.2                        | $2.81 \times 10^{-23}$ |                   | ANXA1, B2M, C3, CCL20, CFB, CP, CTSS, CXCL1, CXCL10, CXCL2, CXCL8, EIF4E, FN1, GBP1, GBP2, GTA1, GTA2, HLA-DR, HMOX1, HSPA5, ICAM1, IDO1, IFIT3, IFGBP3, ISG15, ISG20, LCN2, MIF, MX1, NAMPT, NFkB2, NQO1, P4HB, PCNA, PIgr, PSMB10, PSMB8, PSMB9, PSME2, RAN, SERPINA3, SERPINB2, SOD2, STAT1, TAP2, TGM2, TNFAIP2, TYP, UBD, UBE2L6, VIM, XDH BST2, CXCL10, CXCL8, DDX58, GBP1, GBP5, HLA-B, HLA-C, IFIH1, IFIT2, IFIT3, IFITM1, ISG15, ISG20, MX1, OAS3, PML, PSMB9, STAT1, UBE2L6 |
| IFNL1 cytokine     | Activated     | 4.4                        | $1.84 \times 10^{-21}$ |                   | BST2, CXCL10, CXCL8, DDX58, GBP1, GBP5, HLA-B, HLA-C, IFIH1, IFIT2, IFIT3, IFITM1, ISG15, ISG20, MX1, OAS3, PML, PSMB9, STAT1, UBE2L6 |
| Interferon alpha   | Group         | Activated                  | 5.4                | $6.66 \times 10^{-21}$ | APOL2, B2M, BST2, C3, CXCL1, CXCL10, CXCL2, CXCL8, DDX58, GBP1, GBP2, GBP5, HLA-B, HLA-C, ICAM1, IDO1, IFIH1, IFIT2, IFIT3, IFITM1, ISG15, ISG20, LAP3, MX1, OAS3, PML, PSMB8, PSMB9, RNF213, STAT1, TAP1, TAP2, TAPBP, TYP, UBE2L6, WARS1 |
| CD40LG cytokine    | Activated     | 3.2                        | $8.58 \times 10^{-21}$ |                   | ACTA1, ACTG1, ATP1A3, ATP5F1B, B2M, CCL20, CLU, CXCL1, CXCL10, CXCL2, CXCL8, ICAM1, IDO1, IFIH1, IFIT2, IFIT3, IFITM1, ISG15, LMAN2, MAP2K3, MIF, MSH2, MX1, NAMPT, NFkB2, PML, PSMB10, PSMB8, PSMB9, PSME1, PSME2, SOD2, STAT1, TAP1, TAP2, TGM2, TNFAIP2, TYP, UBD B2M, C3, CCL20, CFB, CXCL1, CXCL10, CXCL2, CXCL8, ERAP2, FN1, GBP1, GTA1, GTA2, HLA-B, HMOX1, ICAM1, IFGBP2, ISG15, MIF, NAMPT, NFkB2, PKM, PRDX2, PSMB9, REG3A, SOD2, TAP1, TAP2, TAPBP, TGM2, TNFAIP2, TPTM, UBD, VIM |
| RELA transcription regulator | Activated | 2.4                        | $1.77 \times 10^{-20}$ |                   | ACAT1, ACTA1, ANXA4, ARF3, BOP1, CLU, CXCL1, CXCL10, CXCL2, CXCL8, DDX58, FN1, GAPDH, GBP2, GBP4, H2AZ1, HDAC2, HLA-B, HMOX1, ICAM1, IFIH1, IFIT2, IFIT3, ISG15, LMAN2, MAP2K3, MIF, MSH2, MX1, NAMPT, NFkB2, PML, PSMB10, PSMB9, PSME1, PSME2, STAT1, TAP1, TAP2, UBE2L6, WARS1 |
| IRF7 transcription regulator | Activated | 4.7                        | $5.05 \times 10^{-20}$ |                   | ACAT1, ACTA1, ACTG1, ATP1A3, ATP5F1B, B2M, CCL20, CLU, CP, CXCL1, CXCL10, CXCL2, CXCL8, DDX58, FN1, GAPDH, GBP2, GBP4, HMOX1, HSPA5, ICAM1, IDO1, IFIH1, IFIT2, IFIT3, ISG15, LMAN2, MAP2K3, MIF, MSH2, MX1, NAMPT, NFkB2, PML, PSMB10, PSMB9, PSME1, PSME2, STAT1, TAP1, TAP2, UBE2L6, WARS1 |
| APP other          | Activated     | 3.6                        | $1.64 \times 10^{-19}$ |                   | ACTA1, ACTA1, ANXA4, ARF3, BOP1, CLU, CXCL10, CXCL8, DDX58, DEK, EIF4E, FN1, GAPDH, GBP2, GBP4, H2AZ1, HDAC2, HLA-B, HMOX1, ICAM1, IFIH1, IFIT2, ISG15, LMAN2, MAP2K3, MIF, MSH2, MX1, MYOB, NQO1, PCNA, PDCD4, PKM, PRDX2, PRDX5, PRDX6, PSME1, RA, RNF213, RPS6, SCG5, SOD2, SPTAN1, SQSTM1, TAGLN, TNFAIP2, TPI1, TTR, TUBA1A, TXN, UCHL1 |
| Ifnar group        | Activated     | 4.3                        | $3.37 \times 10^{-19}$ |                   | B2M, C3, CXCL10, DDX58, GBP2, IDO1, IFIH1, IFIT2, IFIT3, ISG15, ISG20, ISG90, PSMB8, PSMB9, RNF213, STAT1, TAP1, TAP2, TAPBP, UBE2L6, WARS1 |
| MYC transcription regulator | −0.8   | −0.8                       | $5.15 \times 10^{-18}$ |                   | B2M, C3, CXCL10, DDX58, GBP2, IDO1, IFIH1, IFIT2, IFIT3, ISG15, ISG20, ISG90, PSMB8, PSMB9, RNF213, STAT1, TAP1, TAP2, TAPBP, UBE2L6, WARS1
We then assessed if and how the addition of metformin to cytokines modified the above-described scenario. Figure 5A shows the heatmap of the topmost significantly affected canonical pathways, when comparing the two different treatments. A positive
or negative z-score value indicates the activation or inhibition of canonical pathways, significant for a value >2 and < −2, respectively. Metformin reduced cytokine-promoted activation of IL-6, IL-8, and IL-15 signaling; JAK/Stat signaling; mTOR signaling; senescence pathway; HMGB1 signaling; and Gα12/13 signaling. Glycolysis I was also reduced in the presence of metformin. Concerning the upstream regulators, metformin addition reduced the activation induced by cytokines of thrombospondin (THBS4), interleukin 15 (IL-15), interferon epsilon (IFNE), and tumor necrosis factor ligand superfamily member 12 (TNFSF12) and inhibited regulatory-associated protein of mTOR (RAPTOR) (Figure 5B).

Figure 5. Heat map showing the canonical pathways and upstream regulators differentially regulated by cytokines and cytokines plus metformin vs. the respective control samples. Activated (red) or inhibited (blue) canonical pathways (A) and upstream regulators (B) obtained by IPA analysis of human islet differentially regulated proteins in the two different comparisons. The brighter the color, the more intense the change is. Canonical pathways (panel A) were selected based on the highest differences in z-score values.

3.4. Western Blot (WB) Analysis of ERAP2 and IFI30 in Human Islets

The different expression of IFI30 and ERAP2 (proteins involved in antigen processing) in human islets treated with cytokines and cytokines plus metformin was also evaluated using WB analysis. A specific 28 KDa immunoreactive band was detected for IFI30 (Figure 6A), while four specific immunoreactive bands, with apparent weights of 110, 105, 70, and 45 kDa (the first 2 reported in Figure 6A), were detected for ERAP2, corresponding to different isoforms of this protein. In our shotgun experiments, ERAP2 was identified in band 3 of the 1DE gel, corresponding to the highest molecular weight isoforms 1 and 3 of 110 and 105 KDa, respectively. According to the shotgun proteomic analysis, WB showed a significantly higher expression of both IFI30 and ERAP2 in islets treated with cytokines alone than in control samples (Figure 6B,C). The changes induced by the addition of metformin, as observed by proteomic evaluation (significant decrease of IFI30 and significant increase of ERAP2 expression), remained as an apparent, although not significant, trend.
Figure 6. Western blot (WB) analysis was used to evaluate the expression of IFI30 and ERAP2, in both cytokines and cytokines + metformin treated islets compared to control samples. (A) Immunoreactive bands of IFI30 (28KDα), ERAP2 (110KDα), and β-actin, as observed in four independent islet preparations in the three different conditions. (B,C) Violin plots of the normalized OD, reported as mean values ± SEM, for IFI30 (panel B) and ERAP2 (panel C). The dashed line represents the median values, and the dot lines represent the first and the third quartiles. Statistical analysis was performed using a parametric paired t test. * p < 0.05, ** p < 0.01.

4. Discussion

The present study reports the analysis of human pancreatic islets after 48 h exposure to pro-inflammatory cytokines (50 U/mL IL-1β and 1000 U/mL IFN-γ), with or without the presence of metformin, using a multidimensional shotgun proteomics approach. We observed protective effects of metformin on cytokine-induced β-cell functional damage and increased caspase 3/7 activity and investigated the underlying molecular mechanisms by assessing the related proteome modulation.

The previously observed deleterious effects of cytokines on human islet β-cell function and survival [21,58] were confirmed in the present study. Interestingly, the presence of metformin partially prevented β-cell dysfunction and activation of caspase 3/7 (a marker of apoptosis), similarly to the protective action that the compound exerts on human islets exposed to lipoglucotoxicity [28,30] and on islets isolated from type 2 diabetic patients [29]. This suggests that metformin has broad beneficial effects on stressed human islet cells, regardless of the insulting condition, possibly due to pleiotropic, and so far poorly understood, mechanisms.

A few previous studies have investigated the effects of pro-inflammatory cytokines on human islet gene and protein expression [45,46,59–61]. Recently, Nakayasu et al. used tandem mass tags and the label-free technique to study islet proteomics in depth [42]. In our study, 3115 proteins were identified in control samples (untreated islets), of which 3014 were also reported by Nakayasu et al. [42], indicating the good reproducibility of the
results between the two approaches. After cytokine exposure, we found that 244 proteins were differentially expressed compared with the control islets, mainly pertaining to the cytoskeleton, immune response signaling, apoptosis signaling, energy metabolism, protein metabolism, and RNA metabolism. Of them, 57 were also reported in ref [42]. All but two of these 57 proteins were modulated in the same direction in both studies and were mostly upregulated compared to control islets.

Interestingly, the addition of metformin to cytokine-treated islets inhibited several canonical pathways and upstream regulators related to inflammation, such as interleukin and HMGB1 (high mobility group protein B1) signaling and IL15 [62,63]. An anti-inflammatory effect of metformin has been described in a few models, including pancreatic islets [64–67]. The mechanisms by which metformin dampens inflammation are still unclear. However, the drug can reduce oxidative stress [29,68], which is linked to the promotion of inflammatory processes [69,70]. Accordingly, in metformin-treated islets, we also found a significantly increased expression of proteins involved in the defense against oxidative stress [71,72], such as glutathione S-transferase α1 and 2 (GSTA1 and 2); thioredoxin 1 (TRX1); and peroxiredoxins 2, 3, and 5 (PRDX2, 3, and 5). The peroxiredoxin/thioredoxin antioxidant system has been described in rodent β-cell lines and pancreatic islets as a relevant protective mechanism against oxidative damage [73–76]. The specific role of each peroxiredoxin is still debated. PRDX1 has been recently described as the main isoform involved in protection against hydrogen peroxide and peroxynitrite [77]. Interestingly, we presently observed that the two mitochondrial peroxiredoxin isoforms, PRDX3 and PRDX5, resulted as upregulated in metformin-treated islets, suggesting that the mechanism of metformin action in mitochondria could go beyond the inhibition of complex I [78].

Metformin was also able to reduce cytokine-induced immune response through the inhibition of pathways such as the systemic lupus erythematosus in T cell signaling pathway, PKCθ signaling in T lymphocytes, role of NFAT in regulation of the immune response, iCOS-iCOSL signaling in T helper cells, and acute phase response signaling. Some immune-suppressive action of metformin via modulation of T lymphocyte activity has been described [79]. In our study, the addition of metformin to cytokines reduced the expression of components of major histocompatibility complex (MHC) class I antigen presentation, such as HLA class I histocompatibility antigen B alpha chain and C alpha chain (HLA-B and HLA-C) and beta-2-microglobulin (B2M), which is critical for the expression of functional HLA-I molecules on the cell surface [80,81]. These changes might have protective effects on β-cells in T1D, by decreasing the presentation of b-cell β-cell neoantigens for the immune system [82]. Conversely, the expression of HLA-A and G α chains increased in the islets exposed to cytokines and metformin. HLA-G, a non-classical HLA class I molecule having immunomodulatory properties, has previously been found to be constitutively expressed in human pancreatic islets [83] and has been reported to be involved in the attenuation of autoimmune and inflammatory processes [84].

Our proteomic data also showed variations in the expression of IFI30 and ERAP2, enzymes involved in antigen processing [85,86] and that are required for peptide binding to MHC class I antigens and for generating MHC class II- restricted epitopes from disulfide bond-containing proteins, respectively. IFI30 has been implicated in the pathogenesis of some autoimmune diseases, and genetic polymorphisms of ERAP2 and its paralog ERAP1 are associated with increased susceptibility to autoimmune/chronic inflammatory disorders [87,88]. In agreement with findings previously reported with different models [89,90], we observed that cytokines induced overexpression of both enzymes in human islets, which was confirmed by Western blot analysis. Interestingly, the shotgun proteomics results showed significantly decreased expression of IFI30 and increased expression of ERAP2 when metformin was added to the cytokines, which was tendentially confirmed by Western blot analysis.

Among the proteins exclusively upregulated in metformin-treated islets, calcium/calmodulin-dependent serine protein kinase (CASK) and transcription factor JUNB deserve a special mention, since previous work has implicated them in protecting β cells against
cytokines-induced damage [91,92]. CASK, which plays a role in synaptic transmembrane protein anchoring and ion channel trafficking, seems to be also involved in insulin secretion from β cells [93]. It has been reported that IL-1β reduces CASK expression in INS-1 cells and rat islets, while its overexpression counteracts the cytokine-induced β cell dysfunction, by improving insulin secretion [91]. Although, in our experiments, we did not detect a decrease of CASK expression following cytokine exposure, the addition of metformin induced the expression of this protein kinase, suggesting a possible protective mechanism of the drug at this level. Furthermore, it has been shown that the proinflammatory cytokines IL-1β and INF-γ induce an initial and transient upregulation of JUNB in INS-1E cells [92] and that JUNB overexpression reduces cytokine-induced β cell death [94]. Our data therefore suggest that metformin could contribute to keeping active a self-defense system that is otherwise transient.

Another effect of metformin on cytokine-treated islets inferred from our proteomics results is the inhibition of the upstream regulator RAPTOR (regulatory-associated protein of mTOR) and hence the mTOR (serine/threonine-protein kinase mTOR) signaling pathway. It is known that metformin downregulates mTOR signaling through either 5′-adenosine monophosphate–activated protein kinase (AMPK)-dependent- or independent mechanisms [95,96]. We found a decreased expression of proteins involved in mTOR activity, specifically eukaryotic translation initiation factor 4E (eIF-4E), a regulator of translation, and the 40S ribosomal proteins S3, S6, and S9, involved in protein synthesis [97]. Interestingly, mTOR inhibition is associated with increased autophagic fluxes [98], which, in turn, could favor the function and survival of stressed β-cells [99–102].

Of interest, the senescence pathway was also activated by cytokine-treatment and inhibited by metformin addition. The beneficial action of metformin in mitigating aging hallmarks has previously been reported [95]. Notably, cellular senescence has been identified as a key process in both T1D and T2D development [103–105]. In a murine model of pT2D, it was found that insulin resistance accelerates β-cell senescence, while removal of senescent β-cells (senolysis) improves β-cell function and glucose homeostasis, leading to a better disease outcome [103]. Moreover, it has been reported that during T1D development a subset of β-cells apparently acquire the senescent phenotype and thus contribute to immune-mediated β-cell destruction [104].

This study has some limitations. Although the concentration of metformin that we used is in the therapeutic range, the actual levels of the drug in the pancreatic islet microenvironment in vivo are currently unclear. In addition, pancreatic islets are heterogeneous within the same pancreas and between subjects [38,106], and it is unknown if cytokines and metformin have different effects on islets from different individuals. Nevertheless, the islet functional and proteomics results were generated with cutting edge methodologies applied in experienced laboratories and analyzed using strict statistical assessment. This allowed confirming and supporting data from previous studies and, more importantly, to add new knowledge to the field.

In conclusion, the present study shows, for the first time, that metformin prevents, at least in part, the deleterious actions of pro-inflammatory cytokines on human β-cell function and islet cell caspase 3/7 activation, which is accompanied by several modifications of the islet proteome. These modifications include pathways involved in inflammation, immunity, mTOR signaling, and cellular senescence, all known to impact β-cell health. This evidence suggests that metformin, a widely used drug for the treatment of T2D, might be repurposed for β-cell protection in early T1D.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/cells11152465/s1. Table S1: List of total proteins found in isolated human islets. Table S2: List of differentially expressed proteins after cytokine treatment with respect to controls (in blue color those exclusive of either condition). Table S3: List of differentially expressed proteins after cytokine plus metformin treatment with respect to control (in blue color those exclusive of either condition). Table S4: List of differentially expressed proteins in common between the
two different comparisons and their direction. Table S5. List of differentially expressed proteins in common with Nakayasu ES et al. [42].

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**References**

1. American Diabetes Association. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes—2022. *Diabetes Care* 2021, 45, S17–S38.

2. International Diabetes Federation. Available online: https://idf.org/ (accessed on 7 July 2022).

3. Eizirik, D.L.; Pasquali, L.; Cnop, M. Pancreatic β-cells in type 1 and type 2 diabetes mellitus: Different pathways to failure. *Nat. Rev. Endocrinol.* 2020, 16, 349–362. [CrossRef] [PubMed]

4. Halban, P.A.; Polonsky, K.S.; Bowden, D.W.; Hawkins, M.A.; Ling, C.; Mather, K.J.; Powers, A.C.; Rhodes, C.J.; Sussel, L.; Weir, G.C. β-Cell Failure in Type 2 Diabetes: Postulated Mechanisms and Prospects for Prevention and Treatment. *Diabetes Care* 2014, 37, 1751–1758. [CrossRef] [PubMed]

5. Davies, M.J.; D’Alessio, D.A.; Fradkin, J.; Kernan, W.N.; Mathieu, C.; Mingrone, G.; Rossing, P.; Tsapas, A.; Wexler, D.J.; Buse, J.B.; Management of Hyperglycemia in Type 2 Diabetes. 2018. A Consensus Report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetes Care* 2018, 41, 2669–2701. [CrossRef]

6. Draznin, B.; Aroda, V.R.; Bakris, G.; Benson, G.; Brown, F.M.; Freeman, R.; Green, J.; Huang, E.; Isaacs, D.; Kahan, S.; et al. Pharmacologic Approaches to Glycemic Treatment: Standards of Medical Care in Diabetes-2022. *Diabetes Care* 2022, 45 (Suppl. S1), S125–S143.

7. Bailey, C.J. Metformin: Historical overview. *Diabetologia* 2017, 60, 1566–1576. [CrossRef]

8. Buse, J.B.; Wexler, D.J.; Tsapas, A.; Rossing, P.; Mingrone, G.; Mathieu, C.; D’Alessio, D.A.; Davies, M.J. 2019 Update to: Management of Hyperglycemia in Type 2 Diabetes. 2018. A Consensus Report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetes Care* 2020, 43, 487–493. [CrossRef]

9. Adeva-Andany, M.M.; Ranal-Muino, E.; Fernandez-Fernandez, C.; Pazos-Garcia, C.; Vila-Altesor, M. Metabolic Effects of Metformin in Humans. *Curr. Diabetes Rev.* 2019, 15, 328–339. [CrossRef]

10. Foretz, M.; Guigas, B.; Viollet, B. Understanding the glucoregulatory mechanisms of metformin in type 2 diabetes mellitus. *Nat. Rev. Endocrinol.* 2019, 15, 569–589. [CrossRef]

11. Glossmann, H.H.; Lutz, O.M.D. Pharmacology of metformin—An update. *Eur. J. Pharmacol.* 2019, 865, 172782. [CrossRef]

12. Zheng, J.; Xiao, K.-L.; Chen, L.; Wu, C.; Hu, X.; Zeng, T.; Chen, X.-Q.; Li, W.-J.; Deng, X.; Li, H.; et al. Insulin sensitizers improve the GLP-1 secretion and the amount of intestinal L cells on high-fat-diet-induced catch-up growth. *Nutrition* 2017, 32, 457–467. [CrossRef]

13. Cameron, A.R.; Morrison, V.; Levin, D.; Mohan, M.; Forteath, C.; Beall, C.; McNeilly, A.; Balfour, D.J.; Savinko, T.; Wong, A.K.; et al. Anti-Inflammatory Effects of Metformin Irrespective of Diabetes Status. *Circ. Res.* 2016, 119, 652–665. [CrossRef] [PubMed]

14. Kanigur Sultuybek, G.; Soydas, T.; Yenmis, G. NF-κB as the mediator of metformin’s effect on ageing and ageing-related diseases. *Clin. Exp. Pharmacol. Physiol.* 2019, 46, 413–422. [CrossRef] [PubMed]

15. Zilov, A.V.; Abdelaziz, S.I.; Alshammary, A.; Al Zahraei, A.; Amir, A.; Khailil, S.H.A.; Brand, K.; Elkafrawy, N.; Hassoun, A.A.; Jahed, A.; et al. Mechanisms of action of metformin with special reference to cardiovascular protection. *Diabetes/Metabolism Res. Rev.* 2019, 35, e3173. [CrossRef] [PubMed]

16. Todd, J.A. Etiology of Type 1 Diabetes. *Immunity* 2010, 32, 457–467. [CrossRef]
17. Eizirik, D.L.; Sammeth, M.; Bouckenooghe, T.; Bottu, G.; Sisino, G.; Igoillo-Esteve, M.; Ortis, F.; Santin, I.; Colli, M.L.; Barthson, J.; et al. The hu-man pancreatic islet transcriptome: Expression of candidate genes for type 1 diabetes and the impact of pro-inflammatory cytokines. PLoS Genet. 2012, 8, e1002552. [CrossRef]

18. Ghiasi, S.M.; Dahllof, M.S.; Osmai, Y.; Osmai, M.; Jakobsen, K.K.; Aivazidis, A.; Tyberg, B.; Perruzza, L.; Prause, M.; Christensen, D.P.; et al. Regulation of the β-cell inflammasome and contribution to stress-induced cellular dysfunction and apoptosis. Mol. Cell. Endocrinol. 2018, 478, 106–114. [CrossRef]

19. Roep, B.O.; Kleijwegt, F.S.; van Halteren, A.G.S.; Bonato, V.; Boggi, U.; Vendrame, F.; Marchetti, P.; Dotta, F. Islet in-flammation and CXCL10 in recent-onset type 1 diabetes. Clin. Exp. Immunol. 2010, 159, 338–343. [CrossRef]

20. Colli, M.L.; Ramos-Rodriguez, M.; Nakayasu, E.S.; Alvelos, M.I.; Lopes, M.; Hill, J.L.E.; Turatsinze, J.V.; Coomans de布拉夏内, A.; Russell, M.A.; Raurell-Vila, H.; et al. An inte-grated multi-omics approach identifies the landscape of interferon-α-mediated responses of human pancreatic beta cells. Nut. Commun. 2020, 11, 2584. [CrossRef]

21. Marselli, L.; Dotta, F.; Piro, S.; Santangelo, C.; Masini, M.; Lupi, R.; Realacci, M.; del Guerra, S.; Mosca, F.; Boggi, U.; et al. Th2 cytokines have a par-tial, direct protective effect on the function and survival of isolated human islets exposed to combined proinflammatory and Th1 cytokines. J. Clin. Endocrinol. Metab. 2001, 86, 4974–4978. [CrossRef]

22. Patané, G.; Piro, S.; Rabuazzo, A.M.; Anello, M.; Vigneri, R.; Purrello, F. Metformin restores insulin secretion altered by chronic exposure to free fatty acids or high glucose: A direct metformin ef-fect on pancreatic beta-cells. Diabetes 2000, 49, 735–740. [CrossRef] [PubMed]

23. Simon-Szabó, L.; Kokes, M.; Mandl, J.; Kéri, G.; Csala, M. Metformin Attenuates Palmitate-Induced Endoplasmic Reticulum Stress, Serine Phosphorylation of IRS-1 and Apoptosis in Rat Insulinoma Cells. PLoS ONE 2014, 9, e97868. [CrossRef] [PubMed]

24. Hashemitarab, M.; Bahramzadeh, S.; Saremy, S.; Nejaddehbashi, F. Glucose plus metformin compared with glucose alone on β-cell function in mouse pancreatic islets. Biomed. Rep. 2015, 3, 721–725. [CrossRef] [PubMed]

25. Lundquist, I.; Ahmed-Saeb, M.; Aaltonen, A.; Salehi, A. Metformin Ameliorates Dysfunctional Traits of Giblicenlamide- and Glucose-Induced Insulin Secretion by Sup-pression of Imposed Overactivity of the Islet Nitric Oxide Synthase-NO System. PLoS ONE 2016, 11, e0156868. [CrossRef] [PubMed]

26. Moon, J.S.; Karunakaran, U.; Elumalai, S.; Lee, I.-K.; Lee, H.W.; Kim, Y.-W. Metformin and pancreatic beta cells. Diabetes Complicat. 2017, 31, 21–30. [CrossRef]

27. Shen, X.; Fan, B.; Hu, X.; Luo, L.; Yan, Y.; Yang, L. Metformin Reduces Lipotoxicity-Induced Me-ta-Inflammation in β-Cells through the Activation of GPR40-PLC-IP3 Pathway. J. Diabetes Res. 2019, 2019, 7602427. [CrossRef] [PubMed]

28. Lupi, R.; Del Guerra, S.; Fierabracci, V.; Marselli, L.; Novelli, M.; Patanè, G.; Boggi, U.; Mosca, F.; Piro, S.; Del Prato, S.; et al. Lipotoxicity in Human Pancreatic Islets and the Protective Effect of Metformin. Diabetes 2002, 51, S134–S137. [CrossRef]

29. Marchetti, P.; Del Guerra, S.; Marselli, L.; Lupi, R.; Masini, M.; Pollera, M.; Bugliani, M.; Boggi, U.; Vistoli, F.; Mosca, F.; et al. Pancreatic islets from type 2 diabetic patients have functional defects and increased apoptosis that are amelio-rated by metformin. J. Clin. Endocrinol. Metab. 2004, 89, 5535–5541. [CrossRef] [PubMed]

30. Masini, M.; Anello, M.; Bugliani, M.; Marselli, L.; Filippini, F.; Boggi, U.; Purrello, F.; Occhipinti, M.; Martinò, L.; Marchetti, P.; et al. Prevention by met-form-in of alterations induced by chronic exposure to high glucose in human islet beta cells is associated with preserved ATP/ADP ratio. Diabetes Res. Clin. Pract. 2014, 104, 163–170. [CrossRef]

31. Zhang, M.; Liu, Y.; Huan, Z.; Wang, Y.; Xu, J. Metformin protects chondrocytes against IL-1β in-duced injury by regulation of the AMPK/NF-κB signaling pathway. Pharmacoe 2020, 75, 632–636.

32. Zhang, J.; Huang, L.; Shi, X.; Yang, L.; Hua, F.; Ma, J.; Zhu, W.; Liu, X.; Xuan, R.; Shen, Y.; et al. Metformin protects against myocardial ischemia-reperfusion injury and cell pyroptosis via AMPK/NLRP3 inflammasome pathway. Aging 2020, 12, 24270–24287. [CrossRef] [PubMed]

33. Sun, J.; Huang, N.; Ma, W.; Zhou, H.; Lai, K. Protective effects of metformin on lipopolysaccharide-induced airway epithelial cell injury via NF-kB signaling inhibition. Mol. Med. Rep. 2019, 19, 1817–1823. [PubMed]

34. Matallana-Surget, S.; Leroy, B.; Wattiez, R. Shotgun proteomics: Concept, key points and data mining. Expert Rev Proteom. 2010, 7, 5–7. [CrossRef] [PubMed]

35. Alberio, T.; Pieroni, L.; Ronci, M.; Banfi, C.; Bongarzone, I.; Bottoni, P.; Brioschi, M.; Caterino, M.; Chinello, C.; Cormio, A.; et al. Toward the Standard-i-ization of Mitochondrial Proteomics: The Italian Mitochondrial Human Proteome Project Ini-tia-tive. J. Proteome Res. 2017, 16, 4319–4329. [CrossRef]

36. Vesci, S.; Ronci, M.; Lanuti, P.; De Leilis, L.; Florio, R.; Bologna, G.; Scotti, L.; Carletti, E.; Brugnoli, F.; Di Bella, M.C.; et al. Integrative proteomic and functional analyses provide novel insights into the action of the repurposed drug candidate nitroxoline in AsPC-1 cells. Sci. Rep. 2020, 10, 2574. [CrossRef]

37. Chatterjee, S.; Khunti, K.; Davies, M.J. Type 2 diabetes. Lancet 2017, 389, 2239–2251. [CrossRef]

38. Walker, J.T.; Saunders, D.C.; Brissova, M.; Powers, A.C. The Human Islet: Mini-Organ With Mega-Impact. Endocr. Rev. 2021, 42, 605–657. [CrossRef]

39. Marchetti, P.; Dotta, F.; Lauro, D.; Purrello, F. An overview of pancreatic beta-cell defects in human type 2 diabetes: Implications for treatment. Regul. Pept. 2008, 146, 4–11. [CrossRef]

40. Zhou, J.-Y.; Dann, G.P.; Liew, C.W.; Smith, R.D.; Kulkarni, R.N.; Qian, W.-J. Unraveling pancreatic islet biology by quantitative proteomics. Expert Rev. Proteom. 2011, 8, 495–504. [CrossRef]
Cells 2022, 11, 2465

41. Sacco, F.; Seelig, A.; Humphrey, S.; Krahmer, N.; Volta, F.; Reggio, A.; Marchetti, P.; Gerdes, J.; Mann, M. Phosphoproteomics Reveals the GS3-PDX1 Axis as a Key Pathogenic Signaling Node in Diabetic Islets. Cell Metab. 2019, 29, 1422–1432.e3. [CrossRef]

42. Nakayasu, E.S.; Syed, F.; Tersey, S.A.; Gritsenko, M.A.; Mitchell, H.D.; Chan, C.Y.; Dirice, E.; Turatsinze, J.-V.; Cui, Y.; Kulkarni, R.N.; et al. Comprehensive Proteomics Analysis of Stressed Human Islets Identifies GDF15 as a Target for Type 1 Diabetes Intervention. Cell Metab. 2020, 31, 363–374. [CrossRef] [PubMed]

43. Marselli, L.; Piron, A.; Suleiman, M.; Colli, M.L.; Yi, X.; Khamis, A.; Carrat, G.R.; Rutter, G.A.; Bugliani, M.; Giusti, L.; et al. Persistent or Transient Human β Cell Dysfunction Induced by Metabolic Stress: Specific Signatures and Shared Gene Expression with Type 2 Diabetes. Cell Rep. 2020, 33, 108466. [CrossRef] [PubMed]

44. Bugliani, M.; Tavarini, S.; Grano, F.; Tondi, S.; Lacerenza, S.; Giusti, L.; Ronci, M.; Maidecchi, A.; Marchetti, P.; Tesi, M.; et al. Protective effects of Stevia rebaudiana extracts on beta cells in lipotoxic conditions. Geol. Rundsch. 2021, 59, 113–126. [CrossRef] [PubMed]

45. Brozzi, F.; Nardelli, T.R.; Lopes, M.; Millard, I.; Barthson, J.; Igoillo-Esteve, M.; Grieco, F.A.; Villate, O.; Oliveira, J.M.; Casimir, M.; et al. Cytokines induce endoplasmic reticulum stress in human, rat and mouse beta cells via different mechanisms. Diabetologia 2015, 58, 2307–2316. [CrossRef]

46. Ramos-Rodriguez, M.; Raurell-Vila, H.; Colli, M.L.; Alvelos, M.I.; Subirana-Granés, M.; Juan-Mateu, J.; Norris, R.; Turatsinze, J.-V.; Nakayasu, E.S.; Webb-Robertson, E.-J.M.; et al. The impact of proinflammatory cytokines on the β-cell regulatory landscape provides in-sights into the genetics of type 1 diabetes. Nat. Genet. 2019, 51, 1588–1595. [CrossRef]

47. Lundh, M.; Bugliani, M.; Dahlby, T.; Chou, D.H.; Wagner, B.; Ghiasi, S.M.; De Tata, V.; Chen, Z.; Nissan Lund, M.; Davies, M.J.; et al. The immunopro-teasome is induced by cytokines and regulates apoptosis in human islets. J. Endocrinol. 2017, 233, 369–379. [CrossRef]

48. Del Guerra, S.; Lupi, R.; Marselli, L.; Masini, M.; Bugliani, M.; Sbrana, S.; Torri, S.; Pollera, M.; Boggi, U.; Mosca, F.; et al. Functional and mo-lecular defects of pancreatic islets in human type 2 diabetes. Diabetes 2005, 54, 727–735. [CrossRef]

49. Marchetti, P.; Bugliani, M.; Lupi, R.; Marselli, L.; Masini, M.; Boggi, U. The endoplasmic retic-ulum in pancreatic beta cells of type 2 diabetes patients. Diabetologia 2007, 50, 2486–2494. [CrossRef]

50. Persaud, S.J.; Liu, B.; Jones, P.M. Functional Analysis of Human Islets of Langerhans Maintained in Culture. Methods Mol. Biol. 2011, 806, 55–71. [CrossRef]

51. Pingitore, A.; Chambers, E.S.; Hill, T.; Ruz-Maldonado, I.; Liu, B.; Bewick, G.; Morrison, D.; Preston, T.; Wallis, G.A.; Tedford, C.; et al. The diet-derived short chain fatty acid propionate improves beta-cell function in humans and stimulates insulin secretion from human islets in vitro. Diabetes, Obes. Metab. 2016, 19, 257–265. [CrossRef]

52. Ciregia, F.; Bugliani, M.; Ronci, M.; Giusti, L.L.; Boldrini, C.C.; Mazzoni, M.R.; Mossuto, S.S.; Grano, F.F.; Cnop, M.; Marselli, L.; et al. Palmitate-induced lipotoxicity alters acetylation of multiple proteins in clonal β cells and human pancreatic islets. Sci. Rep. 2017, 7, 13445. [CrossRef] [PubMed]

53. Ciregia, F.; Giusti, L.; Ronci, M.; Bugliani, M.; Piga, I.; Pieroni, L.; Rossi, C.; Marchetti, P.; Urbani, A.; Lucacchini, A. Glucagon-like peptide 1 pro-tects INS-1E mitochondria against palmitate-mediated beta-cell dysfunction: A proteomic study. Mol. Biosyst. 2015, 11, 1696–1707. [CrossRef] [PubMed]

54. Li, X.; Franz, T.; Atanassov, I.; Colby, T. Step-by-Step Sample Preparation of Proteins for Mass Spectrometric Analysis. Methods Mol. Biol. 2021, 2261, 13–23. [CrossRef]

55. Zhou, Y.; Zhou, B.; Pache, L.; Chang, M.; Khodabakhshi, A.H.; Tanaseichuk, O.; Benner, C.; Chanda, S.K. Metascape provides in-sights into the genetics of type 1 diabetes. Nat. Genet. 2019, 51, 1588–1595. [CrossRef]

56. Krämer, A.; Green, J.; Pollard, J., Jr; Tugendreich, S. Causal analysis approaches in Ingenuity Pathway Analysis. Bioinformatics 2014, 30, 523–530. [CrossRef] [PubMed]

57. Cox, J.; Mann, M. QuantXanpt high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification. Nat. Biotechnol. 2008, 26, 1367–1372. [CrossRef]

58. Wolden-Kirk, H.; Rondas, D.; Bugliani, M.; Korh, F.; Van Lommel, L.; Brusgaard, K.; Christesen, H.T.; Schuit, F.; Proost, P.; Masini, M.; et al. Discover-ery of Molecular Pathways Mediating 1,25-Dihydroxyvitamin D3 Protection Against Cyto-kine-Induced Inflammation and Damage of Human and Male Mouse Islets of Langerhans. Endocrinology 2014, 155, 736–747. [CrossRef]

59. Rondas, D.; Bugliani, M.; D’Hertog, W.; Lage, K.; Masini, M.; Vaelkens, E.; Marchetti, P.; Mathieu, C.; Overbergh, L. Glucagon-Like Peptide-1 Protects Human Islets against Cytokine-Mediated β-Cell Dysfunction and Death: A Proteomic Study of the Pathways Involved. J. Proteome Res. 2013, 12, 4193–4206. [CrossRef]

60. Gonzalez-Duque, S.; Azoury, M.E.; Colli, M.L.; Afonso, G.; Turatsinze, J.-V.; Nigi, L.; Lalanne, A.I.; Sebastiani, G.; Carré, A.; Pinto, S.; et al. Conven-tional and Neo-antigenic Peptides Presented by β Cells Are Targeted by Circulating naïve CD8 T Cells in Type 1 Diabetic and Healthy Donors. Cell Metab. 2018, 28, 946–960.e6. [CrossRef]

61. Wu, W.; Syed, F.; Simpson, E.; Lee, C.-C.; Liu, J.; Chang, G.; Dong, C.; Seitz, C.; Eizirik, D.L.; Mirmira, R.G.; et al. Impact of Proinflammatory Cytokines on Alternative Splicing Patterns in Human Islets. Diabetes 2021, 71, 116–127. [CrossRef]

62. Zhang, J.; Zhang, L.; Zhang, S.; Yu, Q.; Xiong, F.; Huang, K.; Wang, C.-Y.; Yang, P. HMGB1, an innate alarmin, plays a critical role in chronic inflammation of adipose tissue in obesity. Mol. Cell. Endocrinol. 2017, 454, 103–111. [CrossRef] [PubMed]

63. Zhang, J.; Chen, L.; Wang, F.; Zou, Y.; Li, J.; Luo, J.; Khan, F.; Sun, F.; Li, L.; Liu, J.; et al. Extracellular HMGB1 exacerbates auto-immune progression and recurrence of type 1 diabetes by impairing regulatory T cell stability. Diabetologia 2020, 63, 987–1001. [CrossRef] [PubMed]
64. Saisho, Y. Metformin and Inflammation: Its Potential Beyond Glucose-lowering Effect. *Endocrine, Metab. Immune Disord. Drug Targets* **2015**, *15*, 196–205. [CrossRef] [PubMed]

65. Kristófí, R.; Eriksson, J.W. Metformin as an anti-inflammatory agent: A short review. *J. Endocrinol.* **2021**, *251*, R11–R22. [CrossRef]

66. Yang, X.; Xu, Z.; Zhang, C.; Cai, Z.; Zhang, J. Metformin, beyond an insulin sensitizer, targeting heart and pancreatic β cells. *Biochim. Biophys. Acta Mol. Basis Dis.* **2017**, *1863*, 1984–1990. [CrossRef]

67. Huang, H.; Lorenz, B.R.; Zelmanovitz, P.H.; Chan, C.B. Metformin Preserves β-Cell Compensation in Insulin Secretion and Mass Expansion in Prediabetic Nile Rats. *Int. J. Mol. Sci.* **2021**, *22*, 421. [CrossRef]

68. Apostolova, N.; Iannantuoni, F.; Gruevska, A.; Muntane, J.; Rocha, M.; Victor, V.M. Mechanisms of action of metformin in type 2 diabetes: Effects on mitochondria and leukocyte-endothelium inter-actions. *Redox Biol.* **2020**, *34*, 101517. [CrossRef]

69. Vaziri, N.D.; Rodriguez-Iturbe, B. Mechanisms of Disease: Oxidative stress and inflammation in the pathogenesis of hypertension. *Nat. Clin. Pr. Nephrol.* **2006**, *2*, 582–593. [CrossRef]

70. Lugrin, J.; Rosenblatt-Velin, N.; Parapanov, R.; Liaudet, L. The role of oxidative stress during inflammatory processes. *Biol. Chem.* **2014**, *395*, 203–230. [CrossRef]

71. Hayes, J.D.; Flanagan, J.U.; Jowsey, I. Glutathione transferases. *Annu. Rev. Pharmacol. Toxicol.* **2005**, *45*, 51–88. [CrossRef]

72. Perkins, A.; Nelson, K.J.; Parsonage, D.; Poole, L.B.; Karplus, P.A. Peroxiredoxins: Guardians against oxidative stress and modulators of peroxide signaling. *Trends Biochem. Sci.* **2015**, *40*, 435–445. [CrossRef] [PubMed]

73. Hotta, M.; Tashiro, F.; Ikegami, H.; Niwa, H.; Ogihara, T.; Yodoi, J.; Miyazaki, J.-I. Pancreatic β-Cell–specific Expression of Thioredoxin, an Antioxidative and Antiapoptotic Protein, Prevents Autoimmune and Streptozotocin-induced Diabetes. *J. Exp. Med.* **1998**, *188*, 1445–1451. [CrossRef] [PubMed]

74. Yamamoto, M.; Yamato, E.; Shu-Ichi, T.; Tashiro, F.; Ikegami, H.; Yodoi, J.; Miyazaki, J.-I. Transgenic Expression of Antioxidant Protein Thioredoxin in Pancreatic β Cells Prevents Progression of Type 2 Diabetes Mellitus. *Antioxid. Redox Signal* **2008**, *10*, 43–50. [CrossRef] [PubMed]

75. Stancill, J.S.; Broniowska, K.A.; Oleson, B.J.; Naatz, A.; Corbett, J.A. Pancreatic β-cells detoxify H2O2 through the peroxiredoxin/thioredoxin antioxidant system. *J. Biol. Chem.* **2019**, *294*, 4843–4853. [CrossRef]

76. Stancill, J.S.; Corbett, J.A. The Role of Thioredoxin/Peroxiredoxin in the β-Cell Defense Against Oxidative Damage. *Front. Endocrinol.* **2021**, *12*, 718253. [CrossRef] [PubMed]

77. Stancill, J.S.; Happ, J.T.; Broniowska, K.A.; Hogg, N.; Corbett, J.A. Peroxiredoxin 1 plays a primary role in protecting pancreatic β-cells from hydrogen peroxide and peroxynitrite. *Am. J. Physiol. Integr. Comp. Physiol.* **2020**, *318*, R1004–R1013. [CrossRef]

78. Vial, G.; Detaille, D.; Guigas, B. Role of Mitochondria in the Mechanism(s) of Action of Metformin. *Front Endocrinol.* **2019**, *10*, 294. [CrossRef]

79. Ursini, F.; Russo, F.; Pellino, G.; D’Angelo, S.; Chiara, D.; Manfredini, R.; De Giorgi, R. Metformin and Inflammation: Its Potential Beyond Glucose-lowering Effect. *Endocrine, Metab. Immune Disord. Drug Targets* **2015**, *15*, 251. [CrossRef] [PubMed]

80. Wang, D.; Quan, Y.; Yan, Q.; Morales, J.E.; Wetsel, R.A. Targeted Disruption of the β2-Microglobulin Gene Minimizes the Immunogenicity of Human Embryonic Stem Cells. *Stem Cells Transl. Med.* **2015**, *4*, 1234–1245. [CrossRef]

81. Castro-Gutierrez, R.; Alkanani, A.; Michels, A.; Russ, H.A. Protecting Stem Cell Derived Pancreatic Beta-Like Cells From Diabetogenic T Cell Recognition. *Front. Endocrinol.* **2012**, *2*, R70881. [CrossRef]

82. Richardson, S.J.; Rodrigo-Calvo, T.; Gerling, I.C.; Mathews, C.E.; Kaddis, J.S.; Russell, M.A.; Zeissler, M.; Krogvold, L.; Dahl-Jørgensen, K.; et al. Islet cell hyperexpression of HLA class I antigens: A defining feature in type 1 diabetes. *Diabetologia* **2014**, *57*, 582–593. [CrossRef] [PubMed]

83. Castro-Gutierrez, R.; Alkanani, A.; Mathews, C.E.; Michels, A.; Russ, H.A. Protecting Stem Cell Derived Pancreatic Beta-Like Cells From Diabetogenic T Cell Recognition. *Front. Endocrinol.* **2012**, *2*, R70881. [CrossRef]

84. Martin-Villa, J.M.; Vaquero-Yuste, C.; Molina-Alejandre, M.; Juarez, I.; Suárez-Trujillo, F.; López-Nares, A.; Palacio-Gruber, J.; Barrera-Gutiérrez, L.; Fernández-Cruz, E.; Rodríguez-Sainz, C.; et al. HLA-G: Too Much or Too Little? Role in Cancer and Autoimmune Disease. *Front. Immunol.* **2022**, *13*, 796054. [CrossRef] [PubMed]

85. Babaie, F.; Hosseinzadeh, R.; Ebrazeh, M.; Seyfizadeh, N.; Aslani, S.; Salimi, S.; Hemmatzadeh, M.; Azizi, G.; Jadidi-Niaragh, F.; Mohammad, H. The roles of ERPAP1 and ERPAP2 in autoimmunity and cancer immunity: New insights and perspective. *Mol. Immunol.* **2020**, *121*, 7–19. [CrossRef] [PubMed]

86. Arunachalam, B.; Phan, U.T.; Guzeu, H.J.; Cresswell, P. Enzymatic reduction of disulfide bonds in lysosomes: Characterization of a Gamma-interferon-inducible lysosomal thiol reductase (GILT). *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 745–750. [CrossRef]

87. Evanchuk, B.W.; Yates, R.M. The phagosome and redox control of antigen processing. *Free Radic. Biol. Med.* **2018**, *125*, 53–61. [CrossRef]

88. De Castro, J.A.L.; Stratikos, E. Intracellular antigen processing by ERAP2: Molecular mechanism and roles in health and disease. *Hum. Immunol.* **2019**, *80*, 310–317. [CrossRef]

89. Van den Elsen, P.J.; Gobin, S.J.; van Eggermond, M.C.; Peijnenburg, A. Regulation of MHC class I and II gene transcription: Differences and similarities. *Immunogenetics* **1998**, *48*, 208–221. [CrossRef]

90. Compagnone, M.; Cifaldi, L.; Fruci, D. Regulation of ERPAP1 and ERPAP2 genes and their disfunction in human cancer. *Hum. Immunol.* **2019**, *80*, 318–324. [CrossRef]
91. Wang, T.Y.; Liu, X.J.; Xie, J.Y.; Yuan, Q.Z.; Wang, Y. Cask methylation involved in the injury of insulin secretion function caused by interleukin-1β. *J. Cell Mol. Med.* **2020**, *24*, 14247–14256. [CrossRef] [PubMed]

92. Gurzov, E.N.; Ortis, F.; Bakiri, L.; Wagner, E.F.; Eizirik, D.L. JunB Inhibits ER Stress and Apoptosis in Pancreatic Beta Cells. *PLoS ONE* **2008**, *3*, e3030. [CrossRef] [PubMed]

93. Wang, Y.; Lin, H.; Hao, N.; Zhu, Z.; Wang, D.; Li, Y.; Chen, H.; Zhu, Y.; Han, X. Forkhead box O1 mediates defects in palmitate-induced insulin granule exocytosis by downregulation of calcium/calmodulin-dependent serine protein kinase expression in INS-1 cells. *Diabetologia* **2015**, *58*, 1272–1281. [CrossRef] [PubMed]

94. Gurzov, E.N.; Barthson, J.; Marhfour, I.; Ortis, F.; Naamane, N.; Igoillo-Esteve, M.; Mathieu, C.; Kitajima, S.; Marchetti, P.; et al. Pancreatic β-cells activate a JunB/ATF3-dependent survival pathway during inflammation. *Oncogene* **2011**, *31*, 1723–1732. [CrossRef] [PubMed]

95. Kulkarni, A.S.; Gubbi, S.; Barzilai, N. Benefits of Metformin in Attenuating the Hallmarks of Aging. *Cell Metab.* **2020**, *32*, 15–30. [CrossRef] [PubMed]

96. Jaafar, R.; Tran, S.; Shah, A.N.; Sun, G.; Valdearcos, M.; Marchetti, P.; Masini, M.; Swisa, A.; Giacometti, S.; Bernal-Mizrachi, E.; et al. mTORC1-to-AMPK switching underlies β cell metabolic plasticity during maturation and diabetes. *J. Clin. Investig.* **2019**, *129*, 4124–4137. [CrossRef]

97. Morita, M.; Gravel, S.-P.; Hulea, L.; Larsson, O.; Pollak, M.; St-Pierre, J.; Topisirovic, I. mTOR coordinates protein synthesis, mitochondrial activity and proliferation. *Cell Cycle* **2015**, *14*, 473–480. [CrossRef]

98. Kim, Y.C.; Guan, K.-L. mTOR: A pharmacologic target for autophagy regulation. *J. Clin. Investig.* **2015**, *125*, 25–32. [CrossRef] [PubMed]

99. Rivera, J.F.; Costes, S.; Gurlo, T.; Glabe, C.G.; Butler, P.C. Autophagy defends pancreatic β cells from human islet amyloid polypeptide-induced toxicity. *J. Clin. Investig.* **2014**, *124*, 3489–3500. [CrossRef]

100. Sheng, Q.; Xiao, X.; Prasad, K.; Chen, C.; Ming, Y.; Fusco, J.; Gangopadhyay, N.N.; Ricks, D.; Gittes, G.K. Autophagy protects pancreatic beta cell mass and function in the setting of a high-fat and high-glucose diet. *Sci. Rep.* **2017**, *7*, 16348. [CrossRef]

101. Bugliani, M.; Mossuto, S.; Grano, F.; Suleiman, M.; Marselli, L.; Boggi, U.; De Simone, P.; Eizirik, D.L.; Cnop, M.; Marchetti, P.; et al. Modulation of Autophagy Influences the Function and Survival of Human Pancreatic Beta Cells Under Endoplasmic Reticulum Stress Conditions and in Type 2 Diabetes. *Front Endocrinol.* **2019**, *10*, 52. [CrossRef] [PubMed]

102. Lambelet, M.; Terra, L.F.; Fukaya, M.; Meyerovich, K.; Labriola, L.; Cardozo, A.K.; Allagnat, F. Dysfunc-tional autophagy following exposure to pro-inflammatory cytokines contributes to pancreatic β-cell apoptosis. *Cell Death Dis.* **2018**, *9*, 96. [CrossRef] [PubMed]

103. Aguayo-Mazzucato, C.; Andle, J.; Lee, T.B., Jr.; Midha, A.; Talemal, L.; Chipashvili, V.; Hollister-Lock, J.; van Deuren, J.; Weir, G.; Bonner-Weir, S. Acceleration of β Cell Aging Determines Diabetes and Senolysis Improves Disease Outcomes. *Cell Metab.* **2019**, *30*, 129–142.e4. [CrossRef] [PubMed]

104. Thompson, P.J.; Shah, A.; Ntranos, V.; Van Gool, F.; Atkinson, M.; Bhushan, A. Targeted Elimination of Senescent Beta Cells Prevents Type 1 Diabetes. *Cell Metab.* **2019**, *30*, 1045–1060.e10. [CrossRef] [PubMed]

105. Aguayo-Mazzucato, C. Functional changes in beta cells during ageing and senescence. *Diabetologia* **2020**, *63*, 2022–2029. [CrossRef] [PubMed]

106. Benninger, R.K.P.; Kravets, V. The physiological role of β-cell heterogeneity in pancreatic islet function. *Nat. Rev. Endocrinol.* **2021**, *18*, 9–22. [CrossRef]