

The gene networks downstream of IFN- α -induced kinases in human β -cells

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Abstract

Background

Type 1 diabetes (T1D) is a chronic autoimmune disease characterized by the progressive loss of pancreatic β cells. Interferon- α (IFN- α) is a key driver of β -cell autoimmunity in T1D, promoting early β -cell dysfunction and HLA class I overexpression, thereby enhancing β -cell recognition and killing by autoreactive CD8⁺ T cells. IFN- α effects are mediated via JAK1/TYK2/STAT and JNK signaling and these pathways have emerged as potential therapeutic targets. Their relative contribution to IFN- α -induced transcriptional responses, however, remains poorly defined. Here, we performed a comparative analysis of JAK1/2, TYK2, and JNK inhibition in IFN- α -treated human β cells to identify shared and pathway-specific transcriptional programs.

Methods

Human EndoC- β H1 cells were exposed to IFN- α for 24 h in the presence or absence of the JAK1/2 inhibitor baricitinib, the TYK2 inhibitor deucravacitinib, or the JNK inhibitor SP600125. Transcriptional responses were assessed by RNA sequencing, differential gene expression, pathway enrichment, and Rank–Rank Hypergeometric Overlap (RedRibbon) analyses. Selected targets were validated by qPCR, Western blotting, and chemokine secretion assays. The specificity of inhibition was assessed using siRNA-mediated knockdown of JAK1, JAK2, TYK2, or JNK in EndoC- β H1 cells and primary human islets.

Results

IFN- α induced robust activation of inflammatory and immune-related pathways, including upregulation of HLA class I, *B2M*, *CXCL10*, and the stress-associated genes *ATF4*, *XBP1*, *TXNIP*, and *DDIT3*. JAK1/2 and TYK2 inhibition similarly suppressed interferon-stimulated genes and antigen presentation pathways, whereas TYK2 inhibition more effectively reduced antiviral response genes. In contrast, JNK inhibition minimally affected canonical interferon signaling but preferentially attenuated stress-response pathways, including *ATF4* and *DDIT3*. These findings were confirmed using both pharmacological inhibition and siRNAs.

Conclusions

Our findings demonstrate that IFN- α signaling pathways regulate distinct transcriptional programs in human β cells and support combining pathway-specific therapeutic strategies to simultaneously suppress inflammatory and stress responses in T1D.

Plain English Summary

Type 1 diabetes develops when the immune system attacks the insulin-producing beta cells in the pancreas. Before and during this attack, beta cells are exposed to interferon alpha, a signaling protein that normally helps cells defend themselves against viral infections. However, prolonged interferon alpha signaling can also make beta cells more visible to the immune system and cause cellular stress, potentially contributing to their destruction.

We investigated how human beta cells respond to interferon alpha and what happens when three signaling routes downstream of interferon alpha are blocked. We compared inhibitors of JAK1/2, TYK2 and JNK, proteins that transmit signals inside the beta cells. Using RNA sequencing, we examined changes across thousands of genes. We then confirmed key findings in donated human pancreatic islets using molecular and protein-based tests.

Blocking JAK1/2 or TYK2 strongly reduced genes involved in antiviral defense, inflammation, immune recognition and the production of chemical signals that attract immune cells. TYK2 inhibition produced broader effects on the response to interferon alpha. Blocking JNK had less influence on these typical interferon responses but affected selected pathways linked to cellular stress. All three approaches influenced parts of the endoplasmic reticulum stress response, which is activated when cells struggle to maintain protein production and function.

These findings show that the three signaling routes have overlapping but distinct roles in beta cells. Understanding these differences may help researchers choose or combine treatments that reduce harmful inflammation and stress while considering the need to preserve antiviral protection.

Background

The initial stages of type 1 diabetes (T1D) are characterized by a deleterious “dialog” between the pancreatic β -cells and the immune system (1). This dialog is modulated by T1D susceptibility genes that influence both β -cell function and immune responses (2, 3), but the ultimate molecular mechanisms that lead to β -cell destruction remain incompletely understood. To address this question, research has focused on the major signaling networks governing β -cells–immune cells interactions, including JAK/STAT (4–6), NF- κ B signaling (7, 8) and the β -cell cellular stress-response network, particularly endoplasmic reticulum (ER) stress/UPR–ISR signaling (9, 10).

A key cytokine in the early stages of the disease is interferon- α (IFN- α), and several findings support a role for IFN- α in disease initiation and progression: 1. There is histological evidence for the presence of IFN- α and its downstream-induced proteins in the islets of individuals affected by T1D (11, 12); 2. An IFN- α -associated transcriptional signature in the circulation precedes disease (13, 14); 3. Islets isolated from individuals with one or several autoantibodies, but still normoglycemic, or already at stage 3 T1D (i.e. clinical outbreak) show an IFN-induced mRNA signature in the islets, further supporting sustained activation of IFN signaling within the islet microenvironments (15). Two candidate genes for T1D, *BACH2* (5) and *PTPN2* (16), modulate IFN- α signaling in β -cells by providing negative feedback that downregulates the pro-inflammatory effects of the cytokine (5, 15, 17–19).

IFN- α signaling starts by the binding to surface receptors and then activation of two kinases, namely TYK2 and JAK1 (20, 21). These kinases phosphorylate the transcription factors STAT1 and STAT2, which then form a heterotrimeric complex with IRF 7 or 9, migrate to the nucleus and trigger both antiviral and inflammatory responses (22). The initial target of these kinases is a tyrosine located at position 701 (Y701) of STAT1, whose activation peaks around 0.5-4 h, contributing to early migration of the complex to the nucleus and triggering IFN- α responses (5). STAT1 is then phosphorylated at a secondary site, namely serine 727 (S727), that peaks around 24–48 h (5). This step is essential for STAT1 full transcriptional activity and biological function (5, 23). Serine phosphorylation is mediated by IFN- α -triggering of an alternative pathway via the mitogen-activated protein kinase (MAPK) activation, culminating with activation of JNK1 (5, 24).

This increasing understanding of the key role of IFNs on the early stages of T1D has prompted the testing of agents that inhibit either JAK1/2, for instance baricitinib – a drug already in clinical use for other autoimmune diseases such as rheumatoid arthritis, atopic dermatitis and alopecia areata (25, 26) – and TYK2 inhibitors, such as deucravacitinib, which is approved for treatment of plaque psoriasis and is being evaluated in additional autoimmune diseases (27, 28). Baricitinib has been shown to protect human islets *in vitro* against the deleterious effects of IFNs (5, 12) and has already been tested in individuals at stage 3 of T1D (i.e., autoantibody positive and hyperglycemic), preserving C-peptide production for at least 12 months (29). New and larger trials are being initiated with this drug to prevent stage 3 disease and/or delay progressive loss of C-peptide at stage 3 (ClinicalTrials.gov Identifiers: NCT07222332, NCT07222137). Regarding deucravacitinib, it has been shown to partially protect mice against spontaneous or viral-induced diabetes and to prevent the deleterious effects of IFN- α on human islets (30, 31), including the up-regulation of HLA class I (that enables presentation of β -cell neoantigens to CD8 + T-cells, triggering β -cell death), chemokine production (that attracts immune cells to the islets) and induction of ER stress (which will contribute to β -cell apoptosis and generation of neoantigens) (2, 12, 30, 31).

JNK inhibitors also prevent apoptosis induced by the combination of IFNs with cytokines such as IL-1 β or TNF- α *in vitro* (16, 32–34). Recent evidence has identified an IRE1–JNK–p53 axis as a mediator of β -cell apoptosis during excessive unfolded protein response (UPR) activation (35). Aberrant JNK signaling has been mechanistically linked to metabolic disorders such as obesity and type 2 diabetes, a spectrum of fibrotic diseases including idiopathic pulmonary fibrosis and liver fibrosis, chronic inflammatory conditions, and a host of neurodegenerative disorders like Alzheimer's disease and Parkinson's disease (36); thus, a large variety of JNK inhibitors have been generated and evaluated in clinical trials (37, 38). Up to date, however, there are no preclinical or clinical data with the use of these agents for the treatment of T1D.

Against this background, we presently evaluated the transcriptional networks downstream of IFN- α signaling in human β -cells following inhibition of three key inflammatory pathways: JAK1/2, TYK2, and JNK. Because baricitinib inhibits both JAK1 and JAK2 (6, 39, 40), thereby blocking signaling downstream of both the type I interferon receptor (IFNAR1/IFNAR2) and the type II interferon receptor

(IFNGR1/IFNGR2), we additionally examined the effects of IFN- γ stimulation in β -cells treated with baricitinib. Unlike TYK2 inhibition, which selectively targets IFNAR-mediated signaling, baricitinib inhibits signaling initiated by both IFN- α and IFN- γ .

Our findings demonstrate that inhibition of distinct IFN- α downstream signaling pathways results in overlapping yet non-identical molecular responses in human β -cells. We defined the relative contribution of each of these three signaling pathways for selecting and optimizing therapeutic strategies.

Methods

EndoC- β H1 cell culture and transfection

The human insulin-secreting EndoC- β H1 cells were first kindly provided by R. Scharfmann (Institut Cochin, Université Paris Cité, Paris, France) (41) and later purchased from Human Cell Design, Paris, France. Cells were cultured in Dulbecco's modified Eagle medium (DMEM; Gibco, Thermo Fisher Scientific) containing 5.6 mmol/L glucose and supplemented with 2% fatty acid-free BSA fraction V (Roche, Basel, Switzerland), 50 μ mol/L 2-mercaptoethanol (Sigma-Aldrich, St Louis, MO, USA), 10 mmol/L nicotinamide (Calbiochem, Darmstadt, Germany), 5.5 μ g/mL transferrin (Sigma-Aldrich), 6.7 ng/mL sodium selenite (Sigma-Aldrich), and 100 U/mL penicillin plus 100 μ g/mL streptomycin (Lonza, Leusden, Netherlands). Cells were maintained on fibronectin-coated plates as previously described (33). Cell cultures were monthly screened for Mycoplasma contamination using the MycoAlert Mycoplasma Detection Kit (Lonza, Basel, Switzerland) and were consistently Mycoplasma-free. To minimize passage-related variability, cells were thawed from frozen stocks (passages 45–55) and used up to passage 70. For gene silencing experiments, expression of *TYK2*, *JAK1*, *JAK2*, and *JNK1* was knocked down using two siRNAs targeting each gene (Qiagen, Venlo, Netherlands), as previously validated by us (5, 42, 43). AllStars Negative Control siRNA (Qiagen) was used as the nonspecific control siRNA (siCTL). The sequences of all siRNAs are provided in Table S1. Cells were transfected overnight with 30 nM siRNA using Lipofectamine RNAiMAX (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) in Opti-MEM (Gibco, Thermo Fisher Scientific, Waltham, MA, USA), as previously described (44). Following transfection, cells were allowed to recover for 24 h before exposure to human IFN- α (2,000 U/mL) or IFN- γ (1,000 U/mL) (both from PeproTech Inc., Rocky Hill, NJ, USA) for 24 h or 48 h. In parallel, EndoC- β H1 cells were pretreated for 2 h with the selective TYK2 inhibitor deucravacitinib (0.3 μ mol/L), the JAK1/2 inhibitor baricitinib (4 μ mol/L) or the JNK inhibitor SP600125 (20 μ mol/L) (all from Selleck Chemicals, Cologne, Germany), and then exposed to the respective inhibitor in combination with IFN- α (2,000 U/mL) or IFN γ (1,000 U/mL), or to the inhibitor alone, for an additional 24 h or 48 h. The concentrations of deucravacitinib, baricitinib and JNK were selected based on previous studies from our group (5, 12, 45).

Native human islets culture and transfection

Human islets were isolated from non-diabetic organ donors through collagenase digestion followed by density gradient purification (46). Islet isolation was approved by the local ethical committees in Lille, France, and UCL, Belgium, and the use of human pancreatic islets for this study was approved by the Comité d'Éthique Hospitalo-Facultaire Erasme-ULB (Protocol No. P2019/498). Following isolation, islets were characterized as previously reported and cultured in RPMI 1640/M199 medium containing 5/5.5 mM glucose prior to transport to the ULB laboratory. Upon arrival, the islets were dispersed and cultured in Ham's F-10 medium containing 6.1 mM glucose, 10% fetal bovine serum, 2 mM GlutaMAX (all from Gibco, Thermo Fisher Scientific), 50 μ M 3-isobutyl-1-methylxanthine (Sigma-Aldrich), 1% fatty acid-free bovine serum albumin (BSA) fraction V (Roche), and penicillin (50 U/mL) and streptomycin (50 μ g/mL) (Lonza, Verviers, Belgium) (12, 33). The purity of the human β -cell preparations was assessed by immunofluorescence staining for insulin. Each experimental replicate corresponded to a different donor, and donor characteristics are provided in Table S2. The dispersed human islet cells were transfected with siRNAs following the same protocol used for EndoC- β H1 cells. After transfection, cells were allowed to recover for 24 h before exposure to cytokines and/or inhibitors as described above for EndoC- β H1 cells.

RNA extraction and bulk RNA sequencing

Total RNA was extracted from EndoC- β H1 cells using the RNeasy Micro Kit (Qiagen) according to the manufacturer's instructions. RNA concentration and purity were assessed using a NanoDrop spectrophotometer. RNA sequencing libraries were prepared and sequenced at the BRIGHTcore (Brussels Interuniversity Genomics High Throughput Core).

RNA-seq processing was performed as previously described, with modifications (44, 47). Briefly, read quality was assessed using FastQC (v0.11.9), and adapters and low-quality bases were removed using fastp (v0.19.6), discarding reads shorter than 50% of the original read length. Filtered reads were quantified against the human GENCODE release 36 transcriptome (GRCh38.p13) using Salmon (v1.4.0).

Transcript abundance estimates were imported into R using the tximport package and summarized to gene-level counts using the summarizeToGene function with transcript-to-gene annotation obtained from the most recent Ensembl database available through AnnotationHub. When applicable, batch was included in the design formula (design = ~ batch + condition); otherwise, condition alone was included. Pairwise comparisons were performed using the Wald test, and log2 fold changes were moderated using adaptive shrinkage (ashr). P values were adjusted using the Benjamini-Hochberg procedure. Genes with an adjusted P value < 0.05 were considered differentially expressed; no additional minimum fold-change threshold was applied.

Principal component analysis was performed on variance-stabilized counts generated using the variance stabilizing transformation implemented in DESeq2. When batch effects were present, they were removed using the removeBatchEffect function from the limma package for PCA visualization only; batch was retained in the DESeq2 design matrix used for differential expression analysis.

Rank-rank Hypergeometric Overlap (RRHO) analysis

Genome-wide similarities between inhibitor responses were assessed using two-tailed RRHO analysis implemented in RedRibbon, as previously described (48). For each comparison—e.g. TYK2i + IFN- α versus IFN- α , JAK1/2i + IFN- α versus IFN- α , and JNKi + IFN- α versus IFN- α —genes were ranked by log2fold change after restricting the analysis to unique gene symbols shared between the paired datasets. RedRibbon's evolutionary algorithm was used to identify rank thresholds producing the most significant hypergeometric overlap. Concordantly and discordantly regulated genes from the RRHO quadrants were exported for pathway analysis.

Pathway enrichment analysis

Pathway enrichment analysis was performed using pre-ranked Gene Set Enrichment Analysis (GSEA) implemented in the clusterProfiler package. Genes were ranked according to a signed significance score calculated as: $\text{rank} = -\log_{10}(\text{adjusted P value}) \times \text{sign}(\log_2 \text{ fold change})$, thereby integrating both the direction and statistical significance of differential gene expression. Duplicate gene symbols were removed by retaining the highest-ranking entry for each gene prior to enrichment analysis.

Gene sets were obtained from the Molecular Signatures Database (MSigDB) (Broad Institute), using the C2:CP:Reactome and C2:KEGG Medicus collections. In parallel, enrichment analyses were also performed using locally downloaded Reactome and KEGG gene set collections (version 7.2) to ensure reproducibility of the analyses.

Visualization of differential gene expression

To facilitate the comparison of transcriptional responses across experimental conditions, custom dot plots were generated in R using ggplot2. Selected genes were grouped into biologically relevant functional categories.

For each gene, differential expression was obtained from DESeq2 pairwise comparisons between IFN- α -treated cells and untreated controls (IFN- α vs NTC) or between inhibitor-treated cells and IFN- α -treated cells (baricitinib + IFN- α , deucravacitinib + IFN- α , or SP600125 + IFN- α versus IFN- α). Dot color represents the log2 fold change (log2FC), with red indicating gene upregulation and blue indicating gene downregulation relative to the corresponding reference condition. Dot size is proportional to the statistical significance of differential expression and corresponds to $-\log_{10}(\text{adjusted P value})$, such that larger circles represent more statistically significant changes.

Genes with an adjusted P value < 0.05 were considered significantly differentially expressed. The color and size scales were kept identical across all panels to enable direct comparison between treatment conditions.

Total RNA extraction and reverse transcription-quantitative PCR

EndoC- β H1 cells and human islets were washed with PBS, detached and collected in lysis buffer containing 1% 2-mercaptoethanol. Total RNA was isolated using the PureLink™ RNA Micro Scale Kit (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) and reverse-transcribed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA) following the manufacturer's protocols. The quantitative RT-PCR (RT-qPCR) amplification reactions were performed with IQ SYBR Green Supermix (Bio-Rad, Hercules, CA, USA) on a CFX Connect Real-Time PCR Detection System (Bio-Rad). The concentration of the PCR product was determined using the standard curve method (49) and expressed as copies per mL. Target gene expression was normalized to the geometric mean of the housekeeping genes *ACTIN* and *VAPA* mRNAs (50). The primer sequences are provided in Table S3.

Protein extraction and western blot

EndoC- β H1 cells were washed with PBS, detached and total protein was extracted using 1x Laemmli Sample Buffer containing 60 mM Tris-HCl (pH 6.8), 10% glycerol, 2% sodium dodecyl sulfate, 1.5% 2-mercaptoethanol, 1.5% dithiothreitol, and 0.005% bromophenol blue (all from Sigma-Aldrich) and separated on 10% SDS-PAGE. The nitrocellulose membranes were probed using specific primary antibodies: BIP (HSP5), CHOP (DDIT3), GAPDH, JAK1, JAK2, JNK1, PD-L1, Phospho-JNK1, Phospho-STAT1 (Ser727), Phospho-STAT1 (Tyr701), STAT1 and TYK2, all diluted in TBST 0.1% Tween 20 and 5% BSA. The densitometric values were quantified using Image Lab software version 6.1 (Bio-Rad Laboratories; RRID:SCR_014210) or FIJI (FIJI Is Just ImageJ; RRID:SCR_002285). After background subtraction, protein expression levels were normalized to GAPDH or to the corresponding total protein, as appropriate. Detailed information on all primary and secondary antibodies used in this study is provided in Table S4.

CXCL10 secretion by ELISA

The cell culture supernatants from EndoC- β H1 cells (35,000 cells/200 μ L of medium) and human islets (30,000 cells/200 μ L) were collected at the end of the experiments and immediately stored at -80°C until sample processing. The Human CXCL10/IP-10 Immunoassay (Quantikine ELISA kit, R&D Systems, Minneapolis, MN, USA) was used to quantify CXCL10 secretion into the culture medium, and the assay procedure and result calculations were conducted following the manufacturer's recommendations.

Human chemokine array

Secreted chemokines were analyzed using the Human Chemokine Array Kit (R&D Systems, Minneapolis, MN, USA; ARY017) according to the manufacturer's instructions. EndoC- β H1 cells were pretreated for 2 h with baricitinib (4 μ mol/L), deucravacitinib (0.3 μ mol/L), or SP600125 (20 μ mol/L), followed by stimulation with IFN- α (2,000 U/mL) for 48 h in the continuous presence of the respective inhibitor. Conditioned media were collected, centrifuged to remove cellular debris, and incubated overnight with

nitrocellulose membranes pre-spotted with capture antibodies specific for human chemokines. After washing, membranes were incubated with a cocktail of biotinylated detection antibodies, followed by streptavidin-horseradish peroxidase. Chemiluminescent signals were detected. Spot intensities were quantified by densitometry using ImageJ, background-subtracted, averaged between duplicate spots for each analyte, and normalized to the positive reference control spots present on each membrane. Relative chemokine abundance was expressed as fold change compared with non-treated control samples. Analyses were performed using conditioned media from four independent biological experiments (n = 4).

Statistical analysis

Normality of the data was assessed using the Shapiro–Wilk test to ensure Gaussian distribution, and homogeneity of variances was evaluated using Bartlett’s test. Data exhibiting normal distribution and homogeneity of variances were analyzed using one-way analysis of variance (ANOVA) followed by Bonferroni correction for multiple comparisons post-hoc tests. GraphPad Prism version 11 (GraphPad Software; RRID:SCR_002798) was used to perform statistical analysis. The statistical test applied to each experiment is detailed in the corresponding figure legends. In each experiment, n = 1 represents an independent biological observation, such as EndoC- β H1 cells from distinct passages or native human islets from different donors. A P value < 0.05 was considered statistically significant.

Results

JAK1/2, TYK2 and JNK inhibitors differentially modulate the IFN- α -induced transcriptomic response in EndoC- β H1 cells

To investigate the contribution of JAK1/2, TYK2, and JNK signaling pathways to the β -cell response to IFN- α , EndoC- β H1 cells were pretreated for 2 h with the JAK1/2 inhibitor baricitinib (4 μ mol/L), the TYK2 inhibitor deucravacitinib (0.3 μ mol/L), or the JNK inhibitor SP600125 (20 μ mol/L) (12, 16, 45), followed by stimulation with IFN- α (2,000 U/mL) for 24 h (Fig. 1a). Following bulk RNA sequencing, differential gene expression analysis was performed to characterize the transcriptional consequences of each inhibitor.

Principal component analysis (PCA) performed following batch-effect correction for visualization only using the limma package (51) revealed a separation of experimental groups based on both IFN- α exposure and inhibitor treatment (Fig. 1b). PC1 and PC2 accounted for 14% and 9% of the variance, respectively. The samples occupied treatment-associated regions of the PC1–PC2 space, although the distributions of several conditions overlapped. IFN- α -treated and NTC samples separated primarily along PC1. The effects of the inhibitors under basal conditions were evaluated by differential-expression analysis: JAK1/2i, TYK2i and JNKi altered 26, 85 and 64 genes, respectively, relative to NTC. Consistent

with previous studies (12, 15, 52), IFN- α upregulated 2,498 genes and downregulated 2,179 genes as compared to NTC (Fig. 1c, S1).

Baricitinib markedly attenuated the IFN- α -induced transcriptional response (Fig. 1d). Although residual upregulation of selected MHC class I genes remained detectable, the magnitude of induction was substantially reduced compared with IFN- α -treated cells. Direct comparison with IFN- α alone identified 2,566 increased and 2,503 decreased genes following JAK1/2i treatment (Fig. 1c,d). In the JAK1/2i + IFN- α versus NTC comparison, 1385 genes remained increased and 847 remained decreased, corresponding to 2,232 residual DEGs (Fig S1).

Deucravacitinib produced the largest transcriptional change relative to IFN- α alone, with 3,581 increased and 3,668 decreased genes. Following combined TYK2i and IFN- α treatment, 1298 genes remained increased and 928 remained decreased relative to NTC, corresponding to 2,226 residual DEGs.

JNKi increased 1128 genes and decreased 1246 genes relative to IFN- α alone. In contrast to the JAK1/2i and TYK2i conditions, the JNKi + IFN- α comparison retained 2,566 increased and 2,287 decreased genes relative to NTC, corresponding to 4,853 residual DEGs. Thus, the total number of genes differing from NTC was lower as compared to JAK1/2i or TYK2i treatment.

These genome-wide comparisons established differences in the magnitude of the three inhibitor responses.

Over-representation (ORA) analysis identifies distinct biological pathways regulated by JAK1/2, TYK2 and JNK inhibition

We next examined the affected pathways, the overlap between inhibitor signatures and selected gene groups relevant to β -cell inflammation and stress. ORA was performed separately on upregulated and downregulated DEGs using Reactome and KEGG pathway annotations from MSigDB (Fig. 2).

Among genes upregulated by IFN- α relative to NTC, enriched pathways included Signaling by Interleukins, Interferon Signaling, Class I MHC-mediated Antigen Processing and Presentation, Cytokine Signaling, and Toll-like Receptor Cascades (Fig. 2a). Programmed Cell Death and Death Receptor Signaling were also represented. Genes downregulated by IFN- α were enriched in pathways related to cell-cycle progression, translation and metabolism, including M Phase, Mitotic Prometaphase, Translation Initiation, Ribosome and Metabolism of Amino Acids and Derivatives.

Genes reduced by baricitinib relative to IFN- α were enriched in Signaling by Interleukins, Interferon Signaling, Cytokine–Cytokine Receptor Interaction, Lysosome, Toll-like Receptor Cascades and Death Receptor Signaling (Fig. 2b). Genes increased by baricitinib were enriched in Translation, Metabolism of Amino Acids and Derivatives, M Phase, Ribosome, Translation Initiation and RNA Processing (Fig. 2b).

Genes reduced by deucravacitinib as compared to IFN- α alone were enriched in Signaling by Interleukins, Interferon Signaling, Cytokine–Cytokine Receptor Interaction, Toll-like Receptor Cascades and Death Receptor Signaling (Fig. 2c). Genes increased by deucravacitinib were enriched in Translation, Metabolism of Amino Acids and Derivatives, M Phase, Ribosome, Translation Initiation, Signaling by Robo Receptors and RNA Processing (Fig. 2c).

For SP600125, genes increased relative to IFN- α were enriched in Neuronal System, G α Signaling Events, Transmission Across Chemical Synapses, Biological Oxidations, Glycolysis/Gluconeogenesis and Integration of Energy Metabolism (Fig. 2d). Response of EIF2AK1 (HRI) to Heme Deficiency was the only significant pathway among genes decreased by SP600125 (Fig. 2d).

Comparative analysis reveals shared and distinct transcriptional responses to JAK1/2, TYK2 and JNK inhibition

To further investigate the similarities and differences between the effects of JAK1/2, TYK2, and JNK inhibition on the IFN- α -induced β -cell transcriptional response, we performed a comparative analysis of differentially expressed genes identified in the inhibitor-treated conditions relative to IFN- α alone (Fig. 3). The TYK2i, JAK1/2i and JNKi comparisons contained 7,249, 5,468 and 2,374 DEGs, respectively. The JAK1/2i and TYK2i DEG sets shared 82% and 62% genes in common, with 65% and 48% genes in the pairwise-exclusive region and 17% and 13% genes shared with JNKi (Fig. 3a). JNKi shared 40% of DEGs with JAK1/2i and TYK2i together. TYK2i and JNKi shared 22% and 66% of DEGs, respectively, whereas JAK1/2i and JNKi shared 19% and 44% of DEGs. These overlap counts were next studied by threshold-free RRHO analysis.

The three DEG sets intersected at 956 genes, of which 466 were decreased and 441 were increased relative to IFN- α alone by all three inhibitors, corresponding to 907 concordantly regulated genes. The remaining 49 genes changed in different directions among the three comparisons. In the pairwise-exclusive intersections, TYK2i and JNKi shared 607 genes, including 279 concordantly increased and 328 concordantly decreased genes. By comparison, the JAK1/2i–JNKi pairwise-exclusive intersection contained 80 genes, of which 12 were concordantly increased and 17 concordantly decreased. These results identified a larger concordant gene-level overlap between TYK2i and JNKi than between JAK1/2i and JNKi.

To extend the comparison beyond significantly differentially expressed genes, Rank-Rank Hypergeometric Overlap (RRHO) analysis (48) was performed using genome-wide ranked gene lists (Fig. 3b–d). The JAK1/2i–TYK2i comparison showed close concordance in both the up–up and down–down quadrants and had a maximum adjusted RRHO significance score of 5,446.6 (Fig. 3b). The corresponding maximum scores were 2,933.3 for TYK2i–JNKi and 2,916.8 for JAK1/2i–JNKi (Fig. 3c,d). Thus, the ranked-list analysis supported the larger genome-wide concordance between JAK1/2i and TYK2i. Both comparisons involving JNKi also showed concordant up–up and down–down regions.

TYK2i also showed greater gene-level overlap with JNKi than did JAK1/2i. To examine a possible basis for this difference, JNK1 phosphorylation was measured after 1 h of IFN- α exposure (Fig. S2). Deucravacitinib, but not baricitinib, reduced the IFN- α -induced P-JNK1/JNK1 ratio by nearly 50% ($p < 0.05$). Together with previous evidence of IFN- α -induced JAK/STAT-MAPK crosstalk in human β -cells (5), these findings suggest that TYK2-dependent signaling contributes to early JNK1 activation.

To identify biological processes commonly regulated by all three inhibitors, ORA was performed separately on the 441 concordantly increased and 466 concordantly decreased genes in their pairwise-exclusive intersection (Fig. 3e). The concordantly increased genes were enriched in ncRNA processing, ribonucleoprotein-complex biogenesis, ribosome biogenesis, peptide hormone secretion, cellular detoxification and histone acetyltransferase complexes. The concordantly decreased genes were enriched in the unfolded protein response, response to ER stress, PERK-regulated gene expression, ATF4-dependent ER-stress responses, amino-acid transport, intrinsic apoptotic signaling in response to ER stress and cellular responses to IL-1. Thus, despite their different effects on canonical interferon-responsive genes, all three inhibitors shared transcriptional effects on ER-stress, biosynthetic and cellular-stress pathways.

Targeted gene-group analysis distinguishes inhibitor effects on antigen presentation, interferon responses and cellular stress.

Having established the genome-wide differences among the inhibitor responses, we next examined curated gene groups related to antigen processing and presentation, interferon responses, ER stress, apoptosis and proteasome function (Fig. 4).

IFN- α increased all 15 selected MHC class I antigen-processing and presentation genes, including *HLA-A*, *HLA-B*, *HLA-C*, *B2M*, *TAP1*, *TAP2*, *TAPBP*, *ERAP1*, *ERAP2*, *PSMB8*, *PSMB9* and *NLRC5* (Fig. 4a). JAK1/2i and TYK2i significantly reduced all 15 genes relative to IFN- α alone, whereas JNKi significantly affected only one of them. In contrast, most of the selected MHC class II genes showed small or nonsignificant changes following IFN- α exposure and were minimally affected by the inhibitors. Thus, the effects observed in this gene group were concentrated in the MHC class I antigen-processing and presentation machinery.

JAK1/2i and TYK2i each significantly reduced 31 of the 34 selected interferon- and stress-responsive genes, but the magnitude of reduction was generally larger with TYK2i (Fig. 4b). For example, JAK1/2i and TYK2i reduced *ISG15* by $\log_2FC$ -2.95 and -7.70 , *MX1* by -2.48 and -8.62 , and *STAT1* by -0.69 and -3.40 , respectively. JNKi significantly affected six genes. Of these, *STAT2* was directly related to interferon signaling, whereas *SLC7A11*, *PSAT1*, *ASNS*, *PHGDH* and *SHMT2* were associated with amino-acid metabolism and cellular stress. Thus, JNKi had limited effects on the canonical ISG group but affected a subset of metabolic and stress-response genes.

The inhibitor effects were more heterogeneous among the selected ER-stress, general stress-response and apoptosis-related genes (Fig. 4c). IFN- α significantly changed 11 of the 20 genes in this group.

JAK1/2i, TYK2i and JNKi significantly affected 9, 12 and 8 IFN- α -induced genes, respectively. *CHOP* (*DDIT3*) was reduced by JAK1/2i, TYK2i and JNKi, with log₂FC values of - 1.03, - 1.33 and - 0.61, respectively. Unlike the MHC class I and canonical interferon-response groups, the number of affected genes was more similar across the three inhibitors, but the magnitude of the changes varied.

Within the proteasome-related group, IFN- α increased the immunoproteasome components *PSMB8* and *PSMB9* (Fig. 4d). JAK1/2i and TYK2i reduced *PSMB8* by log₂FC - 2.86 and - 5.28, respectively, and *PSMB9* by - 5.34 and - 8.44. JNKi did not significantly change either gene. Several constitutive proteasome subunits were also altered by JAK1/2i or TYK2i, although many had not been significantly increased by IFN- α relative to NTC.

Together, this targeted analysis showed that JAK1/2i and TYK2i affected a similar proportion of selected MHC class I and interferon-response genes, with TYK2i generally producing larger reductions. JNKi had limited effects on these canonical IFN- α -responsive groups but affected selected ER-stress and apoptosis-related genes.

RT-qPCR validation confirms differential effects of JAK1/2, TYK2 and JNK inhibition on IFN- α -responsive genes.

To validate the transcriptomic findings, we quantified by RT-qPCR the expression of key IFN- α -responsive genes involved in antigen presentation and inflammation in EndoC- β H1 cells stimulated with IFN- α for 24 h following pharmacological inhibition of JAK1/2, TYK2 or JNK. In parallel, knockdown of JAK1, JAK2, TYK2, and JNK1 was performed using target-specific siRNAs to confirm the specificity of the inhibitor-mediated effects (Fig. 5; Fig. S3). Based on the RNA-seq analysis, we selected *HLA-A*, *HLA-B*, and *CXCL10*, together with the ER stress-associated genes *BiP* (*HSPA5*), *CHOP* (*DDIT3*) and *ATF4*.

In line with the RNA-seq results, IFN- α induced the expression of *CXCL10*, *HLA-A*, and *HLA-B* by 52-, 17- and 212-fold (Fig. 5a-c). Relative to the IFN- α -induced response, baricitinib reduced *CXCL10*, *HLA-A* and *HLA-B* expression by 97%, 82% and 100%, whereas the corresponding reductions with deucravacitinib were 95%, 74% and 98%. SP600125 changed these genes by 23%, 24%, and 46%, respectively, with statistical significance for *HLA-B* only. The JAK1/2i and TYK2i effects were significant for 3/3 markers, compared with 1/3 following JNKi treatment.

IFN- α increased *BiP*, *CHOP* and *ATF4* mRNA expression by 1.4-, 2.6- and 1.5-fold, respectively (Fig. 5d-f). Baricitinib reduced these responses by 35%, 63%, and 34%, whereas deucravacitinib reduced them by 25%, 51% and 27%. The effects of SP600125 varied between the three genes, with changes of 27%, 56%, and 7%. At the protein level, IFN- α increased BiP abundance after 24 h (Fig. 5g,h). The mean BiP/GAPDH ratio was lower with baricitinib and deucravacitinib than with IFN- α alone, but these differences were not statistically significant. CHOP protein abundance was not significantly altered by IFN- α or inhibitor treatment.

Kinase knockdown by specific siRNAs reproduced several of the effects observed with the drug inhibitors. JAK1 knockdown reduced IFN- α -induced *CXCL10*, *HLA-A* and *HLA-B* expression by 71%, 75%, and 71%, whereas TYK2 knockdown reduced them by 61%, 69%, and 73% (Fig. 5a–c). JNK1 knockdown affected *CXCL10* and *BiP*, but its effects varied across the six measured transcripts and did not uniformly exceed those of SP600125. These results support the involvement of JAK1, TYK2 and JNK1 in the respective transcriptional responses but also identify subtle differences between pharmacological inhibition and kinase knockdown.

In dispersed human islet cells, IFN- α increased *CXCL10*, *HLA-A* and *HLA-B* mRNA expression by 565-, 3.3- and 4.6-fold, respectively (Fig. 5i–k). Baricitinib reduced these responses by 100%, 45%, and 57%, whereas deucravacitinib reduced them by 100%, 48%, and 60%. SP600125 produced changes of 52%, 32%, and 4%. JAK1, TYK2, and JNK1 knockdown reduced *CXCL10* mRNA by around 65%, and JAK1 and TYK2 KD reduced *HLA-A* and *HLA-B* mRNA by around 48%, whereas JNK1 KD reduced these two genes by only 12%. IFN- α also increased CXCL10 secretion from 35 to 26,853 pg/mL, and treatment with baricitinib, deucravacitinib and SP600125 resulted in concentrations of 24, 447, and 11,745 pg/mL, respectively (Fig. 5l). The direction of changes in the secreted CXCL10 matched the mRNA results.

Extending IFN- α exposure to 48 h in EndoC- β H1 cells did not increase the effect of SP600125 on *CXCL10*, *HLA-A* or *HLA-B* expression (Fig. S4). At 48 h, baricitinib reduced these markers by 93%, 89%, and 100%; deucravacitinib by 92%, 84%, and 100%; and SP600125 by 23%, 16%, and 23%. *BiP*, *CHOP* and *ATF4* expression at 48 h increased by 1.13-, 2.8- and 2-fold. Baricitinib and deucravacitinib reduced only *CHOP* mRNA by around 50%. Thus, the limited effect of JNK inhibition on the selected inflammatory genes persisted during prolonged IFN- α exposure.

JAK1/2, TYK2 and JNK inhibitors differentially affect IFN- α -induced chemokine secretion.

To determine whether inhibition of the JAK/STAT or JNK pathways alters IFN- α -induced chemokine secretion in β -cells, we first quantified secretion of the prototypical IFN- α -induced chemokine CXCL10 by ELISA following both 24 h and 48 h IFN- α stimulation in EndoC- β H1 cells (Fig. 6a,b). After 24 h, IFN- α increased CXCL10 secretion by 80-fold, and both baricitinib and deucravacitinib reduced it to the basal level, whereas SP600125 did not have a significant effect.

After 48 h, IFN- α -treated cells released 1,144 pg/mL CXCL10, approximately 2.5-fold more than the mean measured after 24 h (Fig. 6b). Baricitinib and deucravacitinib reduced the 48 h response by 100%, and SP600125 reduced it by 14%. In contrast to the SP600125 result, JNK1 knockdown significantly reduced CXCL10 secretion at 48 h by 75%.

Media collected after 48 h were subsequently analyzed using a membrane-based human chemokine array capable of detecting 31 chemokines (Fig. 6c, Fig. S4e). Of these, 10 were detected above the detection limit: CXCL10, CCL19, CXCL16, CCL2, CCL5, CXCL8, MDK, CXCL12, CXCL4, and FGB (Fig. 6d,e), and 7 of them were significantly increased by IFN- α relative to NTC.

At the transcript level, JAK1/2i + IFN- α and TYK2i + IFN- α conditions showed lower expression than IFN- α alone for eight of the selected chemokine-related genes, whereas the JNKi + IFN- α condition showed lower expression for three (Fig. 6d). At the protein level, the corresponding numbers were 7, 6 and 4, respectively (Fig. 6e). CXCL10 changed from 1.56-fold after IFN- α exposure to 1.15, 1.17 and 1.19 following the JAK1/2i, TYK2i and JNKi treatments. The corresponding values changed from 1.73-fold to 1.23, 1.21 and 1.21 for CXCL16; from 2.64-fold to 1.30, 1.40 and 1.84 for CCL2; and from 1.98-fold to 1.16, 1.27 and 1.57 for CXCL8.

Most analytes changed in the same direction at the 24-h RNA and 48-h secreted-protein levels, although the correspondence was incomplete and CXCL9 and CXCL11 were not detected in conditioned medium.

We also examined the mRNA expression of selected transcriptional regulators associated with chemokine expression (Fig. 6f). JAK1/2i and TYK2i significantly altered 7 and 12 of the displayed genes, respectively, compared with 4 following JNKi. *IRF1* changed by log₂FC values of -1.24, -1.88, *IRF7* by -1.5, -2.92, *IRF9* by -0.48, -2.59 and *STAT3* by -0.29, -0.44 following treatment with JAK1/2i and TYK2i compared to IFN- α alone. Among the NF- κ B/AP-1-associated genes, *JUN*, *RELA*, and *CEBPB* showed changes of -0.6, -0.15, and -1.10, respectively, for all inhibitors compared with IFN- α .

Pharmacological inhibition and knockdown of JAK1/JAK2 reduce IFN- γ -induced inflammatory responses.

We next examined the effects of baricitinib on IFN- γ -induced signaling in both EndoC- β H1 cells and dispersed primary human islets at 24 h (Fig. 7) and 48 h (EndoC- β H1 cells only; Fig. S5). Whereas canonical IFN- α signaling involves JAK1 and TYK2, canonical IFN- γ receptor signaling is mediated by JAK1 and JAK2. In parallel with pharmacological inhibition, JAK1 or JAK2 was partially depleted using target-specific siRNAs to compare baricitinib treatment with kinase knockdown.

In EndoC- β H1 cells, IFN- γ increased *CXCL10*, *HLA-A* and *HLA-B* mRNA expression by 7,667-, 9.7-, and 1,794-fold, respectively (Fig. 7a–c). Baricitinib reduced the IFN- γ -induced expression of these genes by 100%. JAK1 knockdown produced corresponding reductions of 88%, 42% and 71%, whereas JAK2 knockdown reduced the responses by 88%, 52% and 78%. *HLA-A* expression was numerically lower following JAK2 knockdown than following JAK1 knockdown at 24 h; however, after 48 h, the effects of JAK1 and JAK2 knockdown on the three markers were similar, reaching a reduction of around 90%, 60%, and 65%, respectively (Fig. S5a–c).

IFN- γ increased CXCL10 secretion from 5.7 to 3,462 pg/mL per 35K cells after 24 h (Fig. 7g). Baricitinib reduced the secreted CXCL10 concentration by 100%, whereas JAK1 and JAK2 knockdown reduced it by 52% and 84%, respectively.

Among the ER-stress-associated genes, IFN- γ increased *BiP* mRNA by 1.6-fold after 24 h but did not significantly change *CHOP* or *ATF4* expression (Fig. 7d–f). Baricitinib reduced the IFN- γ -induced *BiP* response by 100%, and JAK1 knockdown reduced it by 77%. JAK2 knockdown changed *BiP* expression

by 30%, but this difference was not statistically significant. *CHOP* and *ATF4* remained unchanged following baricitinib treatment or JAK1/JAK2 knockdown. At 48 h, *BiP*, *CHOP* and *ATF4* expression did not change relative to their respective controls (Fig. S5d–f).

In dispersed human islet cells, IFN- γ increased *CXCL10*, *HLA-A* and *HLA-B* mRNA expression by 1,358-, 2.7-, and 3.3-fold, respectively (Fig. 7h–j). Baricitinib reduced these responses by 100%, 95% and 95%. JAK1 knockdown reduced them by 61%, 52%, and 55%, whereas JAK2 knockdown reduced them by 63%, 65%, and 67%. IFN- γ increased CXCL10 secretion from 35 to 45,527 pg/mL per 30K cells, and baricitinib reduced this response by 100% (Fig. 7k). JAK1 and JAK2 knockdown reduced CXCL10 secretion by 68% and 53%, respectively. Thus, baricitinib and partial depletion of either JAK1 or JAK2 reduced the selected IFN- γ -induced inflammatory responses in both experimental models.

Kinase inhibition differentially affects STAT1 Tyr701 and Ser727 phosphorylation downstream of type I and type II interferon signaling.

To further investigate the molecular basis underlying the differential effects of TYK2 and JAK1/2 inhibition on IFN-induced gene expression, we analyzed total STAT1 protein together with phosphorylation of its two major regulatory residues, Tyr701 and Ser727 (Fig. 8). EndoC- β H1 cells were stimulated with IFN- α or IFN- γ in the presence of pharmacological inhibitors or following siRNA-mediated knockdown. PD-L1 was included as an IFN-inducible, STAT1-regulated immune-response protein that modulates interactions between β -cells and immune cells (43).

IFN- α increased total STAT1 and PD-L1 protein expression and increased STAT1 phosphorylation at both Tyr701 and Ser727 (Fig. 8a–c). Relative to IFN- α alone, TYK2 KD reduced the P-Tyr701/STAT1 ratio by 100%, total STAT1/GAPDH by 37% and PD-L1/GAPDH by 74% (Fig. 8a). Deucravacitinib produced corresponding reductions of 100%, 61% and 100%. The P-Ser727/STAT1 ratio did not change following TYK2 knockdown and was reduced by 24% following deucravacitinib treatment, but the change was not statistically significant.

Baricitinib and JAK1 knockdown also reduced IFN- α -induced STAT1 Tyr701 phosphorylation, total STAT1 and PD-L1 (Fig. 8b). Baricitinib reduced the P-Tyr701/STAT1 ratio by 54%, total STAT1/GAPDH by 62% and PD-L1/GAPDH by 100%. The corresponding reductions following JAK1 knockdown were 51%, 26% and 74%. In comparison, the P-Ser727/STAT1 ratio changed by 26% following baricitinib treatment and 23% following JAK1 knockdown, without reaching statistical significance. Thus, the effects of JAK1/TYK2 inhibition on sustained Tyr701 phosphorylation were larger than those on the proportion of STAT1 phosphorylated at Ser727 under IFN- α stimulation.

JNK1 knockdown and SP600125 treatment changed the P-Ser727/STAT1 ratio by 20% and 18%, respectively, but did not change P-Tyr701/STAT1 or PD-L1 expression (Fig. 8c).

IFN- γ increased total STAT1, PD-L1 and phosphorylation of STAT1 at Tyr701 and Ser727 after 24 h (Fig. 8d,e). JAK2 knockdown reduced the P-Tyr701/STAT1 ratio by 64%, P-Ser727/STAT1 by 22%, total

STAT1/GAPDH by 75%, and PD-L1/GAPDH by 86% (Fig. 8d). JAK1 knockdown produced corresponding reductions of 62%, 59%, 27%, and 83%, respectively (Fig. 8e). Baricitinib reduced these four measurements by nearly 100%. Thus, inhibition or knockdown of JAK1/JAK2 reduced sustained STAT1 phosphorylation and PD-L1 accumulation following IFN- γ exposure.

Discussion

The present study compared the effects of JAK1/2, TYK2 and JNK inhibition on the response of human β -cells to IFN- α . The three inhibitors produced overlapping but quantitatively and functionally distinct transcriptional responses. JAK1/2 and TYK2 inhibition affected a larger fraction of the IFN- α -induced inflammatory program than JNK inhibition, particularly genes involved in interferon signaling, MHC class I antigen presentation, immunoproteasome activity and chemokine production. JNK inhibition had smaller effects on these canonical responses but affected a subset of metabolic, ER-stress and apoptosis-related genes. TYK2 inhibition also reduced early IFN- α -induced JNK1 phosphorylation. These findings define both shared and pathway-specific components of the β -cell response to IFN- α .

Our results extend previous studies showing that IFN- α induces antiviral, antigen-presentation, chemokine and stress-response programs in human β -cells (3, 14, 41, 42, 53). Nevertheless, neither JAK1/2 nor TYK2 inhibition fully restored the transcriptome to the untreated state, indicating persistence of inhibitor-insensitive responses and/or incomplete inhibition of the respective signaling cascades.

Notably, JAK1/2 and TYK2 inhibition produced the largest pairwise gene-level and genome-wide ranked-list overlap, although the effects were not identical. Both inhibitors reduced all 15 selected MHC class I antigen-processing and presentation genes and 31 of the 34 selected interferon- and stress-responsive genes. However, TYK2 inhibition generally produced larger reductions in representative ISGs, including *ISG15*, *MX1* and *STAT1*, and left fewer genes differentially expressed relative to non-treated cells. These differences may reflect the specific position of TYK2 within the IFNAR complex, differences in the extent or duration of kinase inhibition, or additional TYK2-dependent signaling effects.

TYK2 inhibition also showed greater transcriptional overlap with JNK inhibition than did JAK1/2 inhibition. A possible explanation for this observation is that deucravacitinib, but not baricitinib, reduces IFN- α -induced JNK1 phosphorylation after 1 h (Fig. S2). These findings identify functional crosstalk between TYK2-dependent IFNAR signaling and early JNK1 activation. This interaction may contribute to the effects of TYK2 inhibition on both canonical interferon-responsive genes and selected stress-associated pathways.

IFN- α predominantly affected MHC class I rather than the selected MHC class II genes. JAK1/2 and TYK2 inhibition reduced *HLA-A*, *HLA-B*, *HLA-C*, *B2M*, *TAP1/2*, *ERAP1/2* and *NLRC5*, together with the immunoproteasome components *PSMB8* and *PSMB9*. Because increased MHC class I expression and immunoproteasome-dependent peptide processing can enhance the presentation of β -cell antigens to autoreactive CD8⁺ T cells, the coordinated reduction of these components may decrease β -cell recognition by the immune system (2, 12, 31, 34).

The three inhibitors differed less clearly in the ER-stress and apoptosis-related gene group. JAK1/2i, TYK2i, and JNKi significantly affected 9, 12, and 8 of the 20 selected genes, respectively, and all three reduced *CHOP* (*DDIT3*) expression. Thus, JNKi seems to preferentially inhibit stress-associated responses. This agrees with previous evidence that JNK contributes to β -cell stress and apoptosis induced by interferons in combination with IL-1 β or TNF- α (16, 32–34).

In parallel with the cellular stress response, chemokine production provided a functional secretory readout of the IFN- α -induced transcriptional response. JAK1/2 and TYK2 inhibition reduced *CXCL10* mRNA and secretion in EndoC- β H1 cells and dispersed human islet cells, in agreement with the central role of IFNAR–JAK–STAT signaling in *CXCL10* induction (2, 54). These findings were extended to 10 additional chemokines, and most of them changed in the same direction at the RNA and secreted-protein levels. JNK1 knockdown reduced *CXCL10* secretion at 48 h, but this effect was not reproduced by SP600125. This discrepancy may reflect inhibition of additional JNK isoforms by SP600125 besides JNK1 (namely JNK2 and JNK3, with JNK3 having protective effects against cytokines in β -cells) (5). These findings highlight selective JNK1 inhibition as a potential therapeutic approach for future clinical trials, as it may preserve physiological functions mediated by other JNK isoforms while selectively targeting disease-relevant signaling. This strategy may be particularly advantageous given the limitations of previous pan-JNK inhibitors, including limited kinase selectivity, off-target effects, and toxicity (36) and the availability of the newly developed lipid nanocarriers that offer the possibility to target agents directly to pancreatic islets (55, 56).

The IFN- γ experiments provided a complementary assessment of baricitinib in a pathway that uses JAK1 and JAK2 rather than TYK2. Baricitinib and partial depletion of JAK1 or JAK2 reduced IFN- γ -induced *CXCL10*, *HLA-A*, and *HLA-B* expression, as well as *CXCL10* secretion, in EndoC- β H1 and dispersed human islet cells. Despite these shared effects on inflammatory and antigen-presentation responses, IFN- α and IFN- γ showed distinct effects on the selected ER-stress markers. IFN- γ increased *BiP* after 24 h but did not significantly alter *CHOP* or *ATF4* at either 24 or 48 h. By comparison, IFN- α affected a broader group of ER-stress-associated genes, with ATF4- and CHOP-related responses becoming more apparent after prolonged exposure.

These differences in downstream responses prompted further examination of STAT1 signaling, a shared component of both IFN- α and IFN- γ pathways. Both IFN- α and IFN- γ increased total STAT1 levels, STAT1 Tyr701 and Ser727 phosphorylation, and PD-L1 protein abundance after 24 h. TYK2 and JAK1/2 inhibition, as well as the corresponding kinase knockdowns, reduced sustained Tyr701 phosphorylation, total STAT1 levels, and PD-L1 expression. These findings provide a molecular link between JAK–STAT pathway inhibition and reduced expression of canonical ISGs, antigen-presentation genes, and *CXCL10*.

The effects on Ser727 phosphorylation were more dependent on the specific cytokine and intervention tested. Under IFN- α stimulation, TYK2 and JAK1/2 inhibition generally produced larger reductions in Tyr701 phosphorylation than in the proportion of total STAT1 phosphorylated at Ser727. JNK1 knockdown and SP600125 preferentially affected Ser727 phosphorylation. Under IFN- γ stimulation,

baricitinib and JAK1/JAK2 knockdown affected both phosphorylation sites. These results suggest that multiple inputs control sustained Ser727 phosphorylation, and their relative contributions differ between type I and type II interferon responses (57, 58).

The reduction in PD-L1, an IFN-inducible and STAT1-regulated immune-response protein in β -cells (43), paralleled the effects of JAK1/TYK2 inhibition on STAT1 and other IFN-responsive proteins, particularly the reduction of HLA class I, immunoproteasome activity and chemokine production that may decrease β -cell visibility and immune-cell recruitment. On the other hand, reducing PD-L1 could remove an inhibitory signal that protects β -cells against T-cells. Previous experiments using co-culture between autoreactive CD8 + T-cells and stem cell-derived islets, however, indicate that HLA class I inhibition overrules PD-L1 inhibition and is sufficient to provide at least partial β -cell protection (31, 59).

The distinct signaling profiles of baricitinib and deucravacitinib may have important translational implications. Baricitinib inhibits JAK1/JAK2-dependent responses downstream of both type I and type II interferon receptors, leading to transient preservation of C-peptide secretion in individuals with recent-onset T1D (29) that is lost one year after arrest of the drug (60). Deucravacitinib selectively targets TYK2-dependent type I interferon signaling and has shown protective effects in preclinical diabetes models and human islets (30, 31). In the present study, TYK2 inhibition produced the largest reduction in several IFN- α -induced inflammatory programs. Type I interferon signaling contributes to antiviral defense, and broad suppression of ISGs may increase susceptibility to infection, although severe viral infections have not been a major issue in clinical trials for other diseases with this drug (61, 62).

Some limitations should be considered when interpreting these findings. First, most experiments were performed in EndoC- β H1 cells, and primary-cell validation was restricted to selected inflammatory markers in dispersed human islets. Second, the experiments are based on one or two time points and thus provide only a snapshot of the biological phenomena studied. Third, we studied single cytokines, i.e. IFN- α and IFN- γ , while the in vivo situation is clearly more complex.

Conclusions

JAK1/2, TYK2 and JNK inhibition produced overlapping but non-identical effects on the IFN- α response of human β -cells. JAK1/2 and TYK2 inhibition targeted the canonical interferon, MHC class I, immunoproteasome and chemokine programs, with TYK2 inhibition generally producing larger changes in representative IFN- α -responsive genes. JNK inhibition had smaller effects on these canonical pathways but modified selected stress-related responses. The partial reduction of JNK1 phosphorylation by TYK2 inhibition identifies crosstalk between receptor-proximal and stress-activated signaling, whereas the STAT1 results demonstrate differential regulation of Tyr701 and Ser727 phosphorylation. Together, these findings provide a framework for comparing β -cell-directed interferon-pathway inhibitors while emphasizing that therapeutic selection must account for effects on inflammatory, antiviral and counter-regulatory responses.

Abbreviations

T1D

type 1 diabetes

IFN

α -Interferon alpha

ER

endoplasmic reticulum

UPR

unfolded protein response

ISR

integrated stress response

MAPK

mitogen-activated protein kinase

TYK2

tyrosine kinase 2

JAK1

Janus kinase 1

JAK2

Janus kinase 2

JNK

c-Jun N-terminal kinase

PCA

principal component analysis

IL

1β -interleukin-1 beta

TNF

α -tumour necrosis factor alpha

IFNAR1/IFNAR2

interferon alpha/beta receptor 1 and interferon alpha/beta receptor 2

IFNGR1/IFNGR2

interferon gamma receptor 1 and interferon gamma receptor 2

IFN

γ -interferon gamma

RRHO

Rank-Rank Hypergeometric Overlap

MHC class I

major histocompatibility complex class I

HLA class I

human leukocyte antigen class I

DEGs
differentially expressed genes
PERK
protein kinase R-like endoplasmic reticulum kinase
BiP
binding immunoglobulin protein
CHOP
C/EBP homologous protein
ATF4
activating transcription factor 4
siRNA
small interfering RNA
CXCL10
C-X-C motif chemokine ligand 10
Tyr701
tyrosine residue at position 701
Ser727
serine residue at position 727

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Author Contribution

OM and JGO - equal contribution to the study. The study was conceptualized by ARR, OM, JGO, and DLE. The funding was acquired by ARR and DLE. Experiments were performed by OM, JGO, PLZ, XY, and ARR. The methodology used was defined by OM, JGO, PLZ, XY, ARR, and DLE. Human pancreatic islets were provided by NIM, AB, FP and JKC. The project was supervised by ARR and DLE. The original draft of this manuscript was written by OM, JGO, ARR and DLE. All the authors were involved in the data analysis and editing of the manuscript and approved the final version of the manuscript. OM, JGO, ARR and DLE have verified the underlying data of this manuscript.

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Data Availability

All data presented in this manuscript are available from the corresponding authors DLE (decio.laks.eizirik@ulb.be) and Olha Melnyk (olha.melnik@ulb.be) upon request. The RNA-seq data will be made publicly available upon article publication via the public repository GEO XXXX

References

1. Engin F, Eizirik DL. Beyond bystanders: How beta cell stress shapes the autoimmune response in T1D. *Sci Transl Med.* 2026;18(842):eaed3762.
2. Eizirik DL, Pasquali L, Cnop M. Pancreatic beta-cells in type 1 and type 2 diabetes mellitus: different pathways to failure. *Nat Rev Endocrinol.* 2020;16(7):349–62.
3. Robertson CC, Elgamal RM, Henry-Kanarek BA, Arvan P, Chen S, Dhawan S, et al. Untangling the genetics of beta cell dysfunction and death in type 1 diabetes. *Mol Metab.* 2024;86:101973.
4. Chaimowitz NS, Ebenezer SJ, Hanson IC, Anderson M, Forbes LR. STAT1 Gain of Function, Type 1 Diabetes, and Reversal with JAK Inhibition. *N Engl J Med.* 2020;383(15):1494–6.
5. Roca-Rivada A, Oliveira JG, Martin-Vazquez E, Coomans de Brachene A, Yi X, Costa-Junior JM, et al. The type 1 diabetes candidate genes PTPN2 and BACH2 regulate the IFN-alpha-induced crosstalk between JAK/STAT and MAPKs pathways in human beta cells. *EBioMedicine.* 2025;120:105932.
6. Su B, Luan ZL, Liu H, Tuomilehto J, Ji X. Janus kinase and signal transducer and activator of transcription inhibitors in type 1 diabetes and immune checkpoint-related diabetes: current status and future perspectives. *Front Immunol.* 2025;16:1571247.

7. Heimberg H, Heremans Y, Jobin C, Leemans R, Cardozo AK, Darville M, et al. Inhibition of cytokine-induced NF-kappaB activation by adenovirus-mediated expression of a NF-kappaB super-repressor prevents beta-cell apoptosis. *Diabetes*. 2001;50(10):2219–24.
8. Eizirik DL, Mandrup-Poulsen T. A choice of death—the signal-transduction of immune-mediated beta-cell apoptosis. *Diabetologia*. 2001;44(12):2115–33.
9. Eizirik DL, Miani M, Cardozo AK. Signalling danger: endoplasmic reticulum stress and the unfolded protein response in pancreatic islet inflammation. *Diabetologia*. 2013;56(2):234–41.
10. Sahin GS, Lee H, Engin F. An accomplice more than a mere victim: The impact of beta-cell ER stress on type 1 diabetes pathogenesis. *Mol Metab*. 2021;54:101365.
11. Foulis AK, Farquharson MA, Meager A. Immunoreactive alpha-interferon in insulin-secreting beta cells in type 1 diabetes mellitus. *Lancet*. 1987;2(8573):1423–7.
12. Colli ML, Ramos-Rodriguez M, Nakayasu ES, Alvelos MI, Lopes M, Hill JLE, et al. An integrated multi-omics approach identifies the landscape of interferon-alpha-mediated responses of human pancreatic beta cells. *Nat Commun*. 2020;11(1):2584.
13. Ferreira RC, Guo H, Coulson RM, Smyth DJ, Pekalski ML, Burren OS, et al. A type I interferon transcriptional signature precedes autoimmunity in children genetically at risk for type 1 diabetes. *Diabetes*. 2014;63(7):2538–50.
14. Lombardi A, Tsomos E, Hammerstad SS, Tomer Y. Interferon alpha: The key trigger of type 1 diabetes. *J Autoimmun*. 2018;94:7–15.
15. Coomans de Brachène A, Alvelos MI, Szymczak F, Zimath PL, Castela A, Marmontel de Souza B, et al. Interferons are key cytokines acting on pancreatic islets in type 1 diabetes. *Diabetologia*. 2024;67(5):908–27.
16. Roca-Rivada A, Marin-Canas S, Colli ML, Vinci C, Sawatani T, Marselli L, et al. Inhibition of the type 1 diabetes candidate gene PTPN2 aggravates TNF-alpha-induced human beta cell dysfunction and death. *Diabetologia*. 2023;66(8):1544–56.
17. Moore F, Colli ML, Cnop M, Esteve MI, Cardozo AK, Cunha DA, et al. PTPN2, a candidate gene for type 1 diabetes, modulates interferon-gamma-induced pancreatic beta-cell apoptosis. *Diabetes*. 2009;58(6):1283–91.
18. Santin I, Moore F, Colli ML, Gurzov EN, Marselli L, Marchetti P, et al. PTPN2, a candidate gene for type 1 diabetes, modulates pancreatic beta-cell apoptosis via regulation of the BH3-only protein Bim. *Diabetes*. 2011;60(12):3279–88.
19. Marroqui L, Santin I, Dos Santos RS, Marselli L, Marchetti P, Eizirik DL. BACH2, a candidate risk gene for type 1 diabetes, regulates apoptosis in pancreatic beta-cells via JNK1 modulation and crosstalk with the candidate gene PTPN2. *Diabetes*. 2014;63(7):2516–27.
20. Plataniias LC. Mechanisms of type-I- and type-II-interferon-mediated signalling. *Nat Rev Immunol*. 2005;5(5):375–86.
21. Schindler C, Plumlee C. Interferons use the JAK-STAT pathway. *Semin Cell Dev Biol*. 2008;19(4):311–8.

22. Zanin N, Viaris de Lesegno C, Lamaze C, Blouin CM. Interferon Receptor Trafficking and Signaling: Journey to the Cross Roads. *Front Immunol*. 2020;11:615603.
23. Wen Z, Zhong Z, Darnell JE. Jr. Maximal activation of transcription by stat1 and stat3 requires both tyrosine and serine phosphorylation. *Cell*. 1995;82(2):241–50.
24. Decker T, Kovarik P. Serine phosphorylation of STATs. *Oncogene*. 2000;19(21):2628–37.
25. Taylor PC, Keystone EC, van der Heijde D, Weinblatt ME, Del Carmen Morales L, Reyes Gonzaga J, et al. Baricitinib versus Placebo or Adalimumab in Rheumatoid Arthritis. *N Engl J Med*. 2017;376(7):652–62.
26. Dogra S, Shah S, Sharma A, Chhabra S, Narang T. Emerging Role of Baricitinib in Dermatology Practice: All We Need to Know! *Indian Dermatol Online J*. 2023;14(2):153–62.
27. Armstrong AW, Gooderham M, Warren RB, Papp KA, Strober B, Thaci D, et al. Deucravacitinib versus placebo and apremilast in moderate to severe plaque psoriasis: Efficacy and safety results from the 52-week, randomized, double-blinded, placebo-controlled phase 3 POETYK PSO-1 trial. *J Am Acad Dermatol*. 2023;88(1):29–39.
28. Strober B, Blauvelt A, Warren RB, Papp KA, Armstrong AW, Gordon KB, et al. Deucravacitinib in moderate-to-severe plaque psoriasis: Pooled safety and tolerability over 52 weeks from two phase 3 trials (POETYK PSO-1 and PSO-2). *J Eur Acad Dermatol Venereol*. 2024;38(8):1543–54.
29. Waibel M, Wentworth JM, So M, Couper JJ, Cameron FJ, MacIsaac RJ, et al. Baricitinib and beta-Cell Function in Patients with New-Onset Type 1 Diabetes. *N Engl J Med*. 2023;389(23):2140–50.
30. Mine K, Nagafuchi S, Akazawa S, Abiru N, Mori H, Kurisaki H, et al. TYK2 signaling promotes the development of autoreactive CD8(+) cytotoxic T lymphocytes and type 1 diabetes. *Nat Commun*. 2024;15(1):1337.
31. Syed F, Ballew O, Lee CC, Rana J, Krishnan P, Castela A, et al. Pharmacological inhibition of tyrosine protein-kinase 2 reduces islet inflammation and delays type 1 diabetes onset in mice. *EBioMedicine*. 2025;117:105734.
32. Kim WH, Lee JW, Gao B, Jung MH. Synergistic activation of JNK/SAPK induced by TNF-alpha and IFN-gamma: apoptosis of pancreatic beta-cells via the p53 and ROS pathway. *Cell Signal*. 2005;17(12):1516–32.
33. Brozzi F, Nardelli TR, Lopes M, Millard I, Barthson J, Igoillo-Estève M, et al. Cytokines induce endoplasmic reticulum stress in human, rat and mouse beta cells via different mechanisms. *Diabetologia*. 2015;58(10):2307–16.
34. Prause M, Berchtold LA, Urizar AI, Hyldgaard Trauelsen M, Billestrup N, Mandrup-Poulsen T, et al. TRAF2 mediates JNK and STAT3 activation in response to IL-1beta and IFNgamma and facilitates apoptotic death of insulin-producing beta-cells. *Mol Cell Endocrinol*. 2016;420:24–36.
35. Sharma RB, Darko C, Wang Y, Castro TA, Lama TD, Gablaski B et al. JNK and p53 cause human and mouse beta cell death during excessive unfolded protein response. *J Clin Invest*. 2026;136(15).
36. Li J, Cao L, Yang Y, Gao Y, Li B, Shao N, et al. The evolution of JNK inhibition: A review on modern targeting strategies. *Eur J Med Chem*. 2026;310:118787.

37. Popmihajlov Z, Sutherland DJ, Horan GS, Ghosh A, Lynch DA, Noble PW et al. CC-90001, a c-Jun N-terminal kinase (JNK) inhibitor, in patients with pulmonary fibrosis: design of a phase 2, randomised, placebo-controlled trial. *BMJ Open Respir Res.* 2022;9(1).
38. Mattos W, Khalil N, Spencer LG, Bonella F, Folz RJ, Rolf JD, et al. Phase 2, Double-Blind, Placebo-controlled Trial of a c-Jun N-Terminal Kinase Inhibitor in Idiopathic Pulmonary Fibrosis. *Am J Respir Crit Care Med.* 2024;210(4):435–43.
39. Shawky AM, Almalki FA, Abdalla AN, Abdelazeem AH, Gouda AM. A Comprehensive Overview of Globally Approved JAK Inhibitors. *Pharmaceutics.* 2022;14(5).
40. Chikhoune L, Poggi C, Moreau J, Dubucquoi S, Hachulla E, Collet A, et al. JAK inhibitors (JAKi): Mechanisms of action and perspectives in systemic and autoimmune diseases. *Rev Med Interne.* 2025;46(2):89–106.
41. Ravassard P, Hazhouz Y, Pechberty S, Bricout-Neveu E, Armanet M, Czernichow P, et al. A genetically engineered human pancreatic beta cell line exhibiting glucose-inducible insulin secretion. *J Clin Invest.* 2011;121(9):3589–97.
42. Marroqui L, Dos Santos RS, Floyel T, Grieco FA, Santin I, Op de Beeck A, et al. TYK2, a Candidate Gene for Type 1 Diabetes, Modulates Apoptosis and the Innate Immune Response in Human Pancreatic beta-Cells. *Diabetes.* 2015;64(11):3808–17.
43. Colli ML, Hill JLE, Marroqui L, Chaffey J, Dos Santos RS, Leete P, et al. PDL1 is expressed in the islets of people with type 1 diabetes and is up-regulated by interferons-alpha and-gamma via IRF1 induction. *EBioMedicine.* 2018;36:367–75.
44. Szymczak F, Alvelos MI, Marin-Canas S, Castela A, Demine S, Colli ML, et al. Transcription and splicing regulation by NLRC5 shape the interferon response in human pancreatic beta cells. *Sci Adv.* 2022;8(37):eabn5732.
45. Coomans de Brachene A, Castela A, Op de Beeck A, Mirmira RG, Marselli L, Marchetti P, et al. Preclinical evaluation of tyrosine kinase 2 inhibitors for human beta-cell protection in type 1 diabetes. *Diabetes Obes Metab.* 2020;22(10):1827–36.
46. Marchetti P, Bugliani M, Lupi R, Marselli L, Masini M, Boggi U, et al. The endoplasmic reticulum in pancreatic beta cells of type 2 diabetes patients. *Diabetologia.* 2007;50(12):2486–94.
47. Yi X, Marmontel de Souza B, Sawatani T, Szymczak F, Marselli L, Marchetti P, et al. Mining the transcriptome of target tissues of autoimmune and degenerative pancreatic beta-cell and brain diseases to discover therapies. *iScience.* 2022;25(11):105376.
48. Piron A, Szymczak F, Papadopoulou T, Alvelos MI, Defrance M, Lenaerts T et al. RedRibbon: A new rank-rank hypergeometric overlap for gene and transcript expression signatures. *Life Sci Alliance.* 2024;7(2).
49. Overbergh L, Valckx D, Waer M, Mathieu C. Quantification of murine cytokine mRNAs using real time quantitative reverse transcriptase PCR. *Cytokine.* 1999;11(4):305–12.
50. Alvelos MI, Szymczak F, Castela A, Marin-Canas S, de Souza BM, Gkantounas I, et al. A functional genomic approach to identify reference genes for human pancreatic beta cell real-time quantitative

- RT-PCR analysis. *Islets*. 2021;13(3–4):51–65.
51. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res*. 2015;43(7):e47.
 52. Szymczak F, Colli ML, Mamula MJ, Evans-Molina C, Eizirik DL. Gene expression signatures of target tissues in type 1 diabetes, lupus erythematosus, multiple sclerosis, and rheumatoid arthritis. *Sci Adv*. 2021;7(2).
 53. Russell MA, Richardson SJ, Morgan NG. The role of the interferon/JAK-STAT axis in driving islet HLA-I hyperexpression in type 1 diabetes. *Front Endocrinol (Lausanne)*. 2023;14:1270325.
 54. Eizirik DL, Colli ML. Revisiting the role of inflammation in the loss of pancreatic β -cells in T1DM. *Nat Reviews Endocrinol*. 2020;16(11):611–2.
 55. Enriquez JR, Zhou Z, Nelson JB, Huang F, Figatner KT, Chakraborty A, et al. Messenger RNA delivery to islet β cells using conjugated lipid nanoparticles. *Cell Rep Med*. 2026;7(3):102634.
 56. Yu D, Zhu Y, Roca-Rivada A, Guo Z, Cheng L, Weng G et al. Engineering Lipid Nanoparticles through Integrated Compositional and Ligand Targeting Enhances β Cell-Directed RNA Delivery. *ACS Nano*. 2026.
 57. Nguyen H, Ramana CV, Bayes J, Stark GR. Roles of phosphatidylinositol 3-kinase in interferon-gamma-dependent phosphorylation of STAT1 on serine 727 and activation of gene expression. *J Biol Chem*. 2001;276(36):33361–8.
 58. Bancerek J, Poss ZC, Steinparzer I, Sedlyarov V, Pfaffenwimmer T, Mikulic I, et al. CDK8 kinase phosphorylates transcription factor STAT1 to selectively regulate the interferon response. *Immunity*. 2013;38(2):250–62.
 59. Chandra V, Ibrahim H, Halliez C, Prasad RB, Vecchio F, Dwivedi OP, et al. The type 1 diabetes gene TYK2 regulates beta-cell development and its responses to interferon-alpha. *Nat Commun*. 2022;13(1):6363.
 60. So M, Waibel M, Couper JJ, Cameron FJ, MacIsaac RJ, Atlas G et al. beta-Cell Function and Diabetes Outcomes 1 Year After Stopping Oral Baricitinib Immunotherapy for Type 1 Diabetes. *Diabetes Care*. 2026.
 61. Bieber T, Katoh N, Simpson EL, de Bruin-Weller M, Thaci D, Torrelo A, et al. Safety of baricitinib for the treatment of atopic dermatitis over a median of 1.6 years and up to 3.9 years of treatment: an updated integrated analysis of eight clinical trials. *J Dermatolog Treat*. 2023;34(1):2161812.
 62. Armstrong AW, Lebwohl M, Warren RB, Sofen H, Imafuku S, Ohtsuki M, et al. Safety and Efficacy of Deucravacitinib in Moderate to Severe Plaque Psoriasis for Up to 3 Years: An Open-Label Extension of Randomized Clinical Trials. *JAMA Dermatol*. 2025;161(1):56–66.

Figures

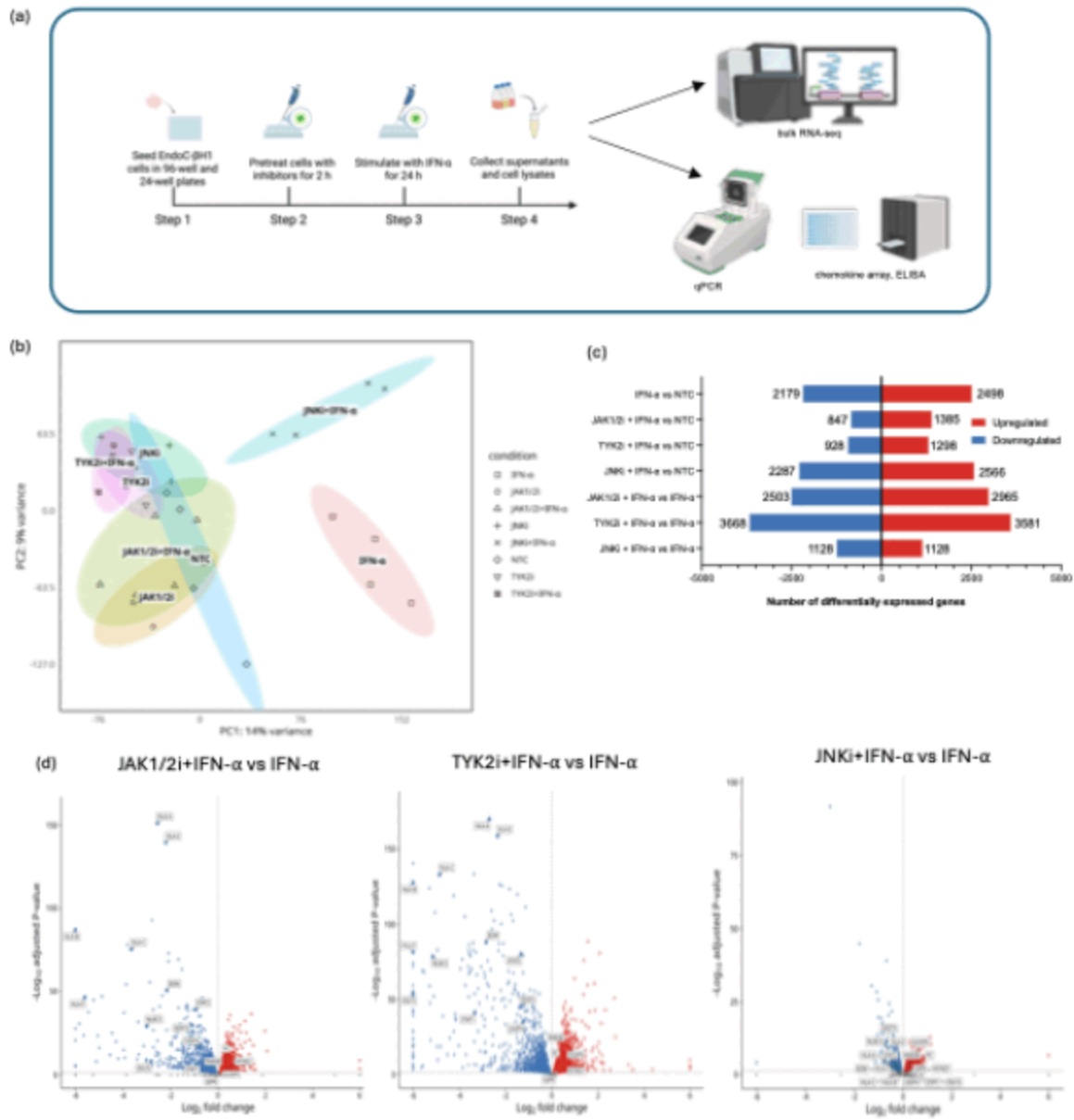


Figure 1

Bulk RNA seq of the EndoC- β H1 cells pretreated with the inhibitors and then exposed to IFN- α .

(a) Experimental workflow. EndoC- β H1 cells were seeded and pretreated or not (non-treated cells, NTC) for 2 h with baricitinib (JAK1/2i: JAK1/2 inhibitor, 4 μ mol/L), deucravacitinib (TYK2i; TYK2 inhibitor, 0.3 μ mol/L), or SP600125 (JNKi; JNK inhibitor, 20 μ mol/L). Cells were subsequently treated or not with IFN- α (2,000 U/mL) for 24 h. Cell lysates and culture supernatants were collected for downstream analyses, including bulk RNA sequencing, qPCR, and chemokine measurements. (b) Principal component analysis (PCA) of bulk RNA-seq data from NTC-, IFN- α -, JAK1/2i-, JAK1/2i+IFN- α -, TYK2i-, TYK2i+IFN- α -, JNKi-, and JNKi+IFN- α -treated EndoC- β H1 cells. Each symbol represents an individual biological replicate. n=4. (c) Numbers of differentially expressed genes for the indicated comparisons. Red bars extending to the right show genes with increased expression, and blue bars extending to the left show genes with decreased expression. Values adjacent to the bars indicate the number of genes in each category. (d)

Volcano plots showing differential gene expression in JAK1/2i+IFN- α , TYK2i+IFN- α and JNKi+IFN- α -treated cells relative to cells treated with IFN- α alone. Each point represents one gene. Red and blue points indicate genes with significantly increased and decreased expression, respectively, whereas gray points indicate genes that did not meet the differential-expression criteria. Horizontal dashed lines indicate the adjusted P-value threshold ($P_{adj} < 0.05$), and vertical dashed lines indicate the fold-change threshold ($|\log_2FC| > 0$). Selected genes are labeled. Differential-expression analyses were based on four independent biological replicates per condition ($n = 4$).

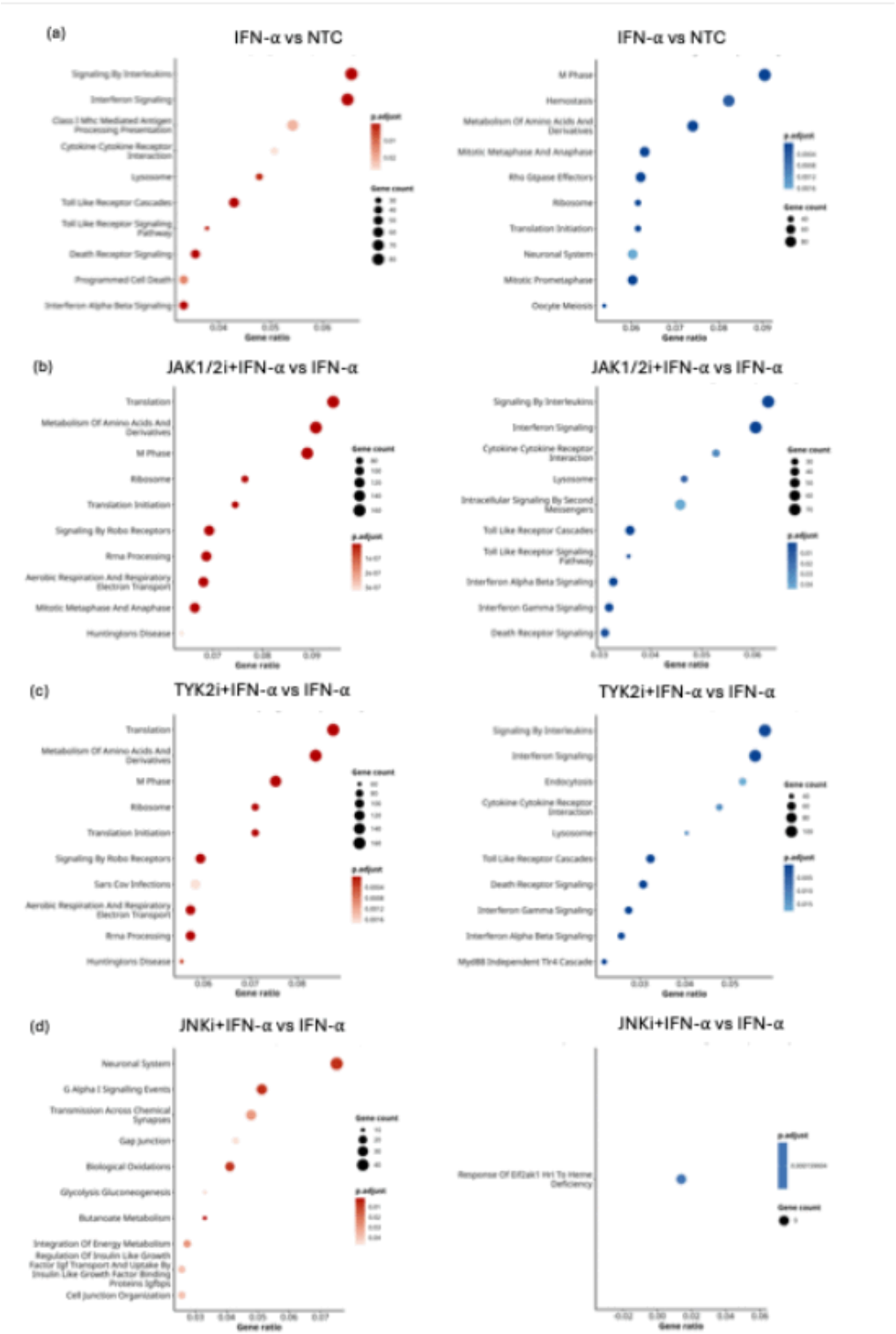


Figure 2

Over-representation analysis of pathways associated with IFN- α stimulation and their modulation by the inhibitors in EndoC- β H1 cells.

(a) Over-representation analysis (ORA) of differentially expressed genes identified in the comparison between IFN- α -treated and non-treated control (NTC) EndoC- β H1 cells. Enriched pathways from the REACTOME and KEGG database upregulated (left) and downregulated (right) are shown. (b) ORA of differentially expressed genes identified in the comparison between Baricitinib+IFN- α -treated and IFN- α -treated cells. Enriched pathways from the REACTOME and KEGG database upregulated (left) and downregulated (right) are shown. (c) ORA of differentially expressed genes identified in the comparison between TYK2i+IFN- α -treated and IFN- α -treated cells. Enriched pathways from the REACTOME and KEGG database upregulated (left) and downregulated (right) are shown. (d) ORA of differentially expressed genes identified in the comparison between JNKi+IFN- α -treated and IFN- α -treated cells. Enriched pathways from the REACTOME and KEGG database upregulated (left) and downregulated (right) are shown. For all panels, ORA was performed using pathway gene sets from the REACTOME and KEGG collections of the MSigDB database. Dot size represents the number of differentially expressed genes associated with each pathway (gene count), dot color indicates the adjusted P value (Padj), and the x-axis shows the gene ratio, defined as the proportion of differentially expressed genes assigned to a given pathway. Pathways are ranked according to enrichment significance, and the top enriched pathways are displayed for each comparison. n=4

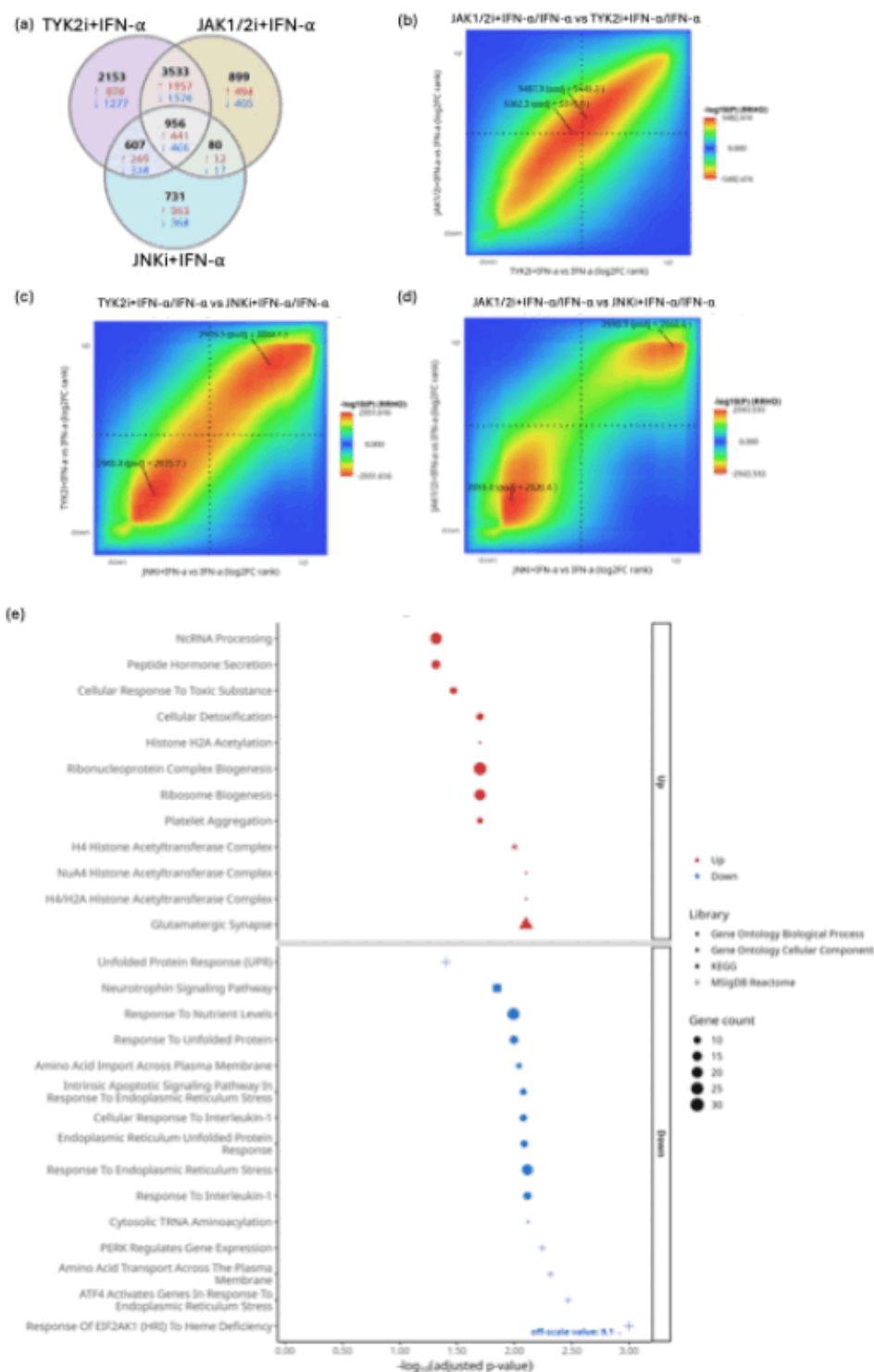


Figure 3

Shared and distinct transcriptional responses to IFN- α pathway inhibition in EndoC- β H1 cells.

(a) Venn diagram showing the overlap of differentially expressed genes identified in the comparisons TYK2i+IFN- α versus IFN- α , JAK1/2i+IFN- α versus IFN- α , and JNKi+IFN- α versus IFN- α . Black numbers indicate the total number of unique or shared DEGs within each region of the diagram, while red and blue

numbers denote the corresponding numbers of upregulated ($\uparrow$) and downregulated ($\downarrow$) genes, respectively. (b–d) Rank–Rank Hypergeometric Overlap (RRHO) analyses comparing transcriptional responses induced by the different inhibitors. RRHO maps were generated using ranked gene lists from the comparisons JAK1/2i+IFN- α versus IFN- α and TYK2i+IFN- α versus IFN- α (b), TYK2i+IFN- α versus IFN- α and JNKi+IFN- α versus IFN- α (c), and JAK1/2i+IFN- α versus IFN- α and JNKi+IFN- α versus IFN- α (d). Color intensity represents the significance of overlap between ranked gene lists, displayed as $-\log_{10}(\text{adjusted P value})$. The number of overlapping upregulated and downregulated genes identified by RRHO is indicated above each heatmap. (e) Over-representation analysis (ORA) of genes commonly regulated by all three inhibitor treatments. Analyses were performed separately for genes commonly upregulated and commonly downregulated in Jak1/2i+IFN- α , TYK2i+IFN- α , and JNKi+IFN- α -treated cells compared with IFN- α -treated cells. Enriched pathways and gene sets were identified using Gene Ontology (GO Biological Process, GO Molecular Function, and GO Cellular Component), KEGG, and MSigDB Reactome databases. The x-axis shows pathway enrichment significance as $-\log_{10}(\text{adjusted P value})$, and dot size indicates the number of genes associated with each pathway. Pathways with $-\log_{10}(\text{adjusted P value}) > 3$ are shown at the x=3 boundary and labeled with their actual value. Colors distinguish pathways enriched among commonly upregulated (red) and commonly downregulated (blue) genes, while symbols indicate the source database.

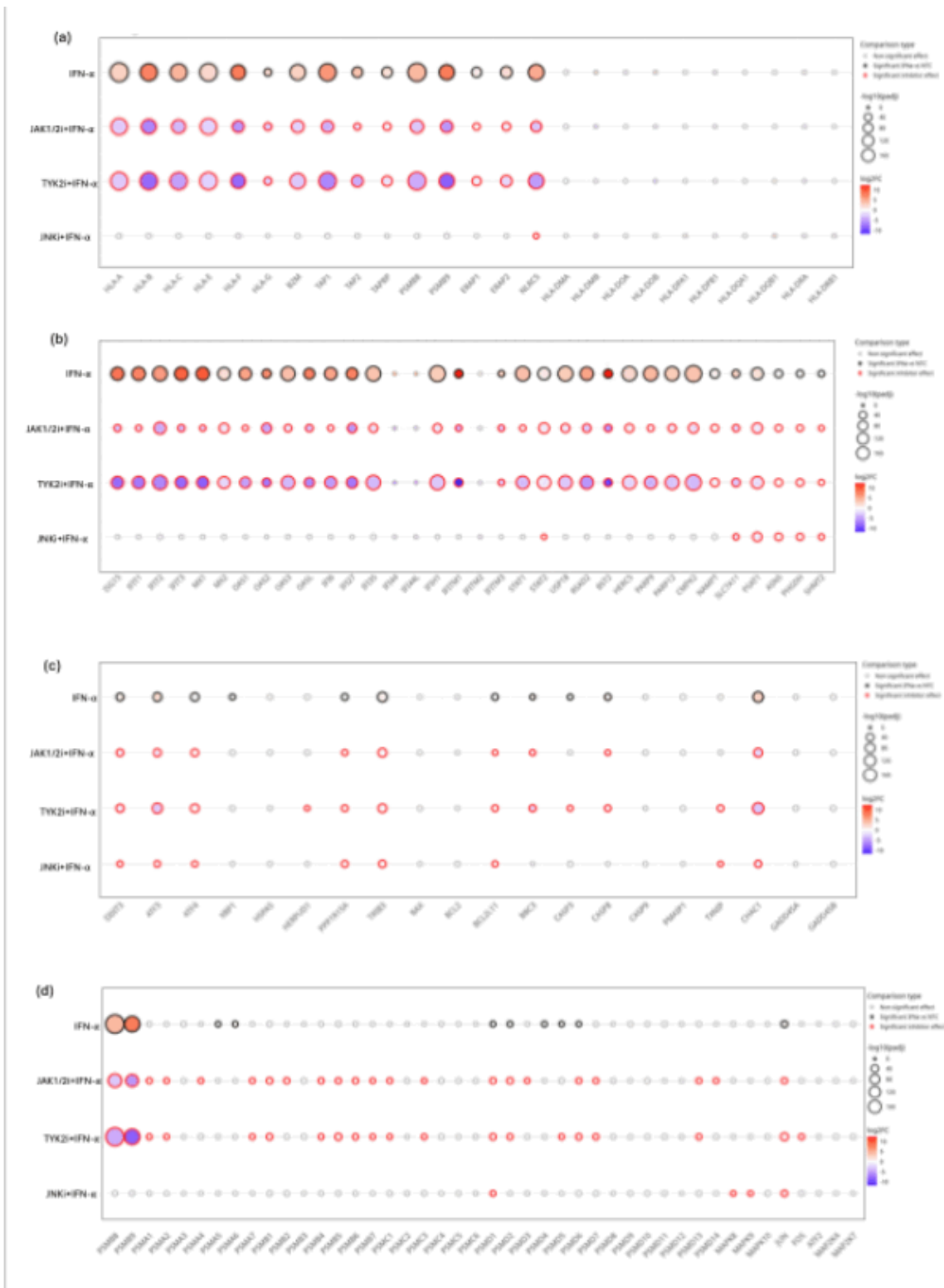


Figure 4

Differential effects of JAK1/2, TYK2, and JNK inhibition on IFN-α-induced gene signatures in EndoC-βH1 cells.

Bubble plots showing the expression changes of selected genes following treatment with baricitinib (JAK1/2i+IFN-α), deucravacitinib (TYK2i+IFN-α), or SP600125 (JNKi+IFN-α) in comparison with IFN-α-

treated cells. The effects of IFN- α alone were compared to non-treated cells. (a) Genes involved in antigen processing and presentation through the major histocompatibility complex (MHC) class I pathway, including HLA class I molecules, peptide transporters, antigen-processing enzymes, and immunoproteasome components. (b) Canonical interferon-stimulated genes (ISGs) and mediators of type I interferon signaling, including antiviral effectors, transcriptional regulators, and inflammatory response genes. (c) Genes associated with ER stress, apoptosis, and cell death pathways, including unfolded protein response regulators, pro-apoptotic factors, and caspase signaling components. (d) Components of the proteasome and immunoproteasome machinery involved in antigen processing and peptide generation for MHC class I presentation. Gene expression changes were derived from RNA-seq differential expression analysis. Bubble size represents the absolute log₂ fold change, while color intensity indicates statistical significance ($-\log_{10}$ adjusted P value). Red outlines denote significantly differentially expressed genes ($P_{adj} < 0.05$), whereas gray circles indicate non-significant changes.

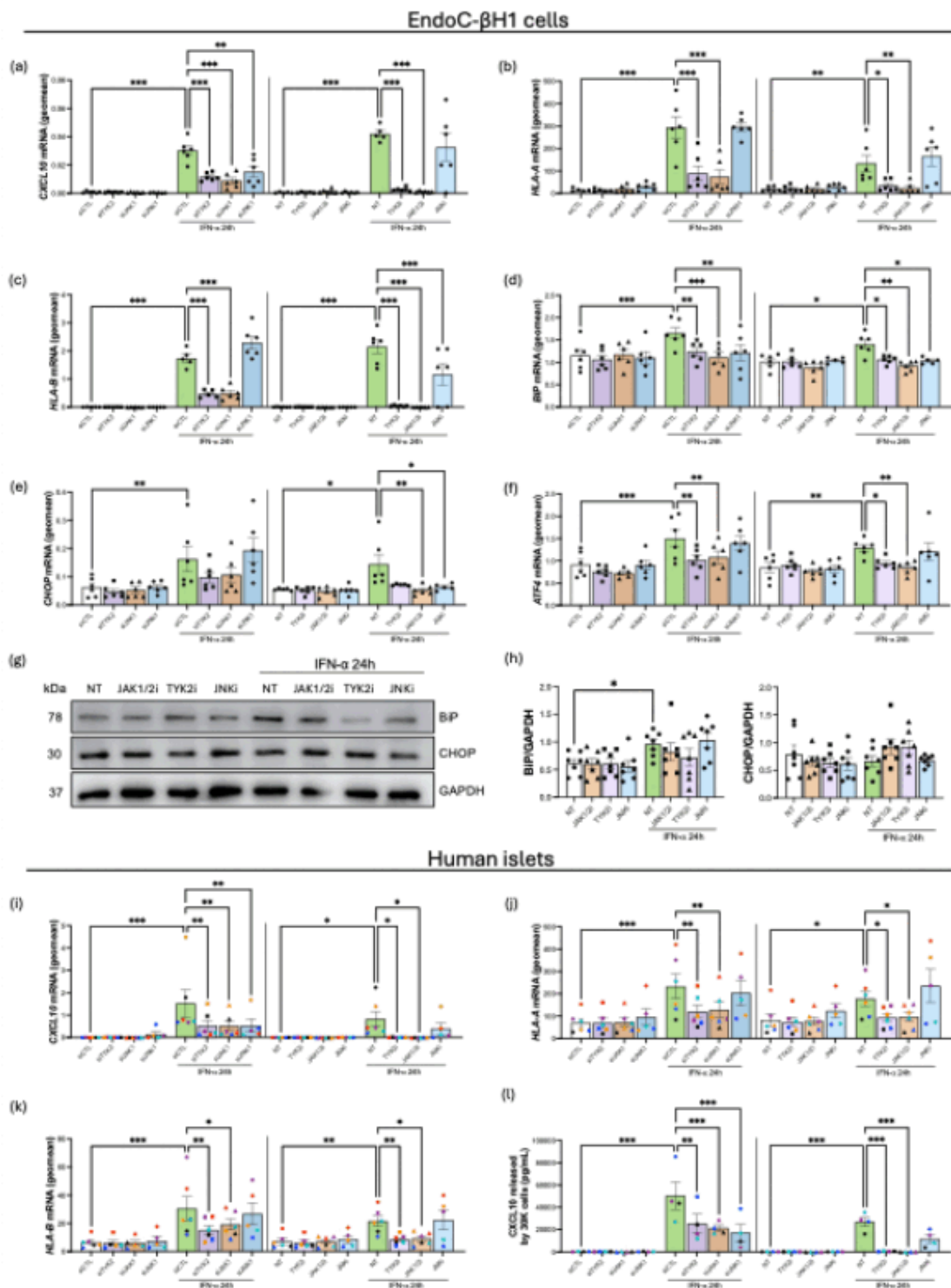


Figure 5

Differential effects of JAK1/2, TYK2 and JNK inhibition and knockdown on IFN- α -induced inflammatory and ER stress responses in EndoC- β H1 cells and primary human islets.

EndoC- β H1 cells (a-h) or human islets (i-l) were transfected with siRNA control (siCTL) or siRNAs targeting TYK2, JAK1 or JNK1. After 24 h of recovery, the cells were left untreated or treated with IFN- α

IFN- α -induced chemokine expression is differentially regulated by JAK1/2, TYK2 and JNK inhibition in EndoC- β H1 cells at the transcript and protein levels.

EndoC- β H1 cells (a,b) were transfected with siRNA control (siCTL) or siRNAs targeting TYK2, JAK1 or JNK1. After 24 h of recovery, the cells were left untreated or treated with IFN- α (2,000 U/mL) for 24 h or 48 h. In parallel, cells were pretreated for 2 h with the selective TYK2 inhibitor deucravacitinib (0.3 μ mol/L), the JAK1/2 inhibitor baricitinib (4 μ mol/L), or the JNK inhibitor SP600125 (20 μ mol/L), and then left untreated or treated with IFN- α (2,000 U/mL) for 24 h or 48 h in the presence of the inhibitors. CXCL10 secretion levels were analyzed by ELISA. Results are $\pm$ SEM of 4 independent experiments. * P < 0.05, ** P < 0.01, and *** P < 0.001. One-way ANOVA followed by Bonferroni correction for multiple comparisons post-hoc test. (c) Representative Human Chemokine Array membranes (R&D Systems, #ARY017) incubated with conditioned media collected from non-treated control (NT), IFN- α -treated. The inset lists the chemokines and other secreted proteins represented on the array. Darker spots indicate higher relative abundance of the corresponding analytes. (d) Heatmap showing RNA-seq-derived $\log_2$ fold-change values of selected chemokine genes in IFN- α -treated EndoC- β H1 cells with and without inhibitor treatment relative to non-treated control (NTC) cells. (e) Heatmap showing fold changes of secreted chemokines detected in conditioned media using the Human Chemokine Array Kit (R&D Systems, #ARY017) under the indicated experimental conditions relative to NTC cells. Protein abundance was quantified by densitometric analysis of array spot intensities. (f) Bubble plot showing RNA-seq-derived expression changes in selected transcription factors associated with chemokine production. The IFN- α condition was compared with NTC, whereas the JAK1/2i+IFN- α , TYK2i+IFN- α and JNKi+IFN- α conditions were compared with IFN- α alone. Bubble size represents $-\log_{10}(P_{adj})$, and fill color represents $\log_2FC$. Black outlines indicate significant effects of IFN- α relative to NTC, red outlines indicate significant inhibitor effects relative to IFN- α alone, and gray outlines indicate nonsignificant changes. Significance was defined as P_{adj} < 0.05. RNA-seq and chemokine-array analyses were based on four independent biological experiments ($n = 4$).

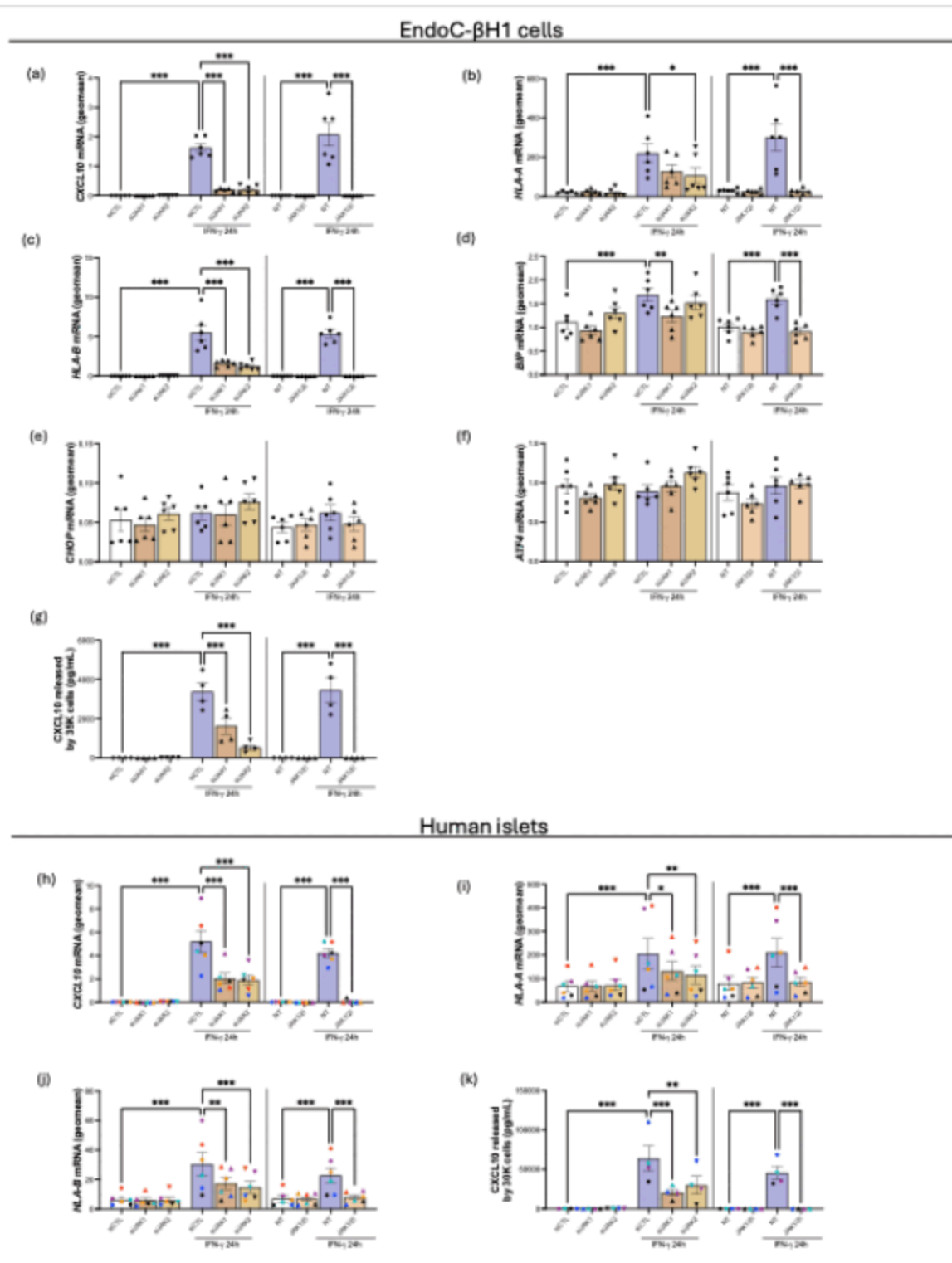


Figure 7

JAK1/2 chemical inhibition or silencing suppresses IFN-γ-induced inflammatory responses in EndoC-βH1 cells and primary human islets.

EndoC-βH1 cells (a-g) or human islets (h-l) were transfected with siRNA control (siCTL) or siRNAs targeting JAK1 or JAK2. After 24 h of recovery, the cells were left untreated or treated with IFN-γ (1,000 U/mL) for 24 h. In parallel, cells were pretreated for 2 h with the JAK1/2 inhibitor baricitinib (4 μmol/L),

and then left untreated or treated with IFN- γ (1,000 U/mL) for 24 h in the presence of the inhibitor. *CXCL10* (a, h), *HLA-A* (b, i), *HLA-B* (c, j), *BIP* (d), *CHOP* (e), and *ATF4* (f) mRNA expression were evaluated by RT-qPCR and normalized by the geometric mean of *ACTIN* and *VAPA*. *CXCL10* secretion levels were analyzed by ELISA (g,i). Results are $\pm$ SEM of 4-6 independent experiments. For the human islets, each color corresponds to a different preparation, black HI 375, blue HIPR-29 and HIPR-24, red HIPR-23 and HIPR-36, purple HIPR-37, orange HI 376, and cyan HIPR-38. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$. One-way ANOVA followed by Bonferroni correction for multiple comparisons post-hoc test.

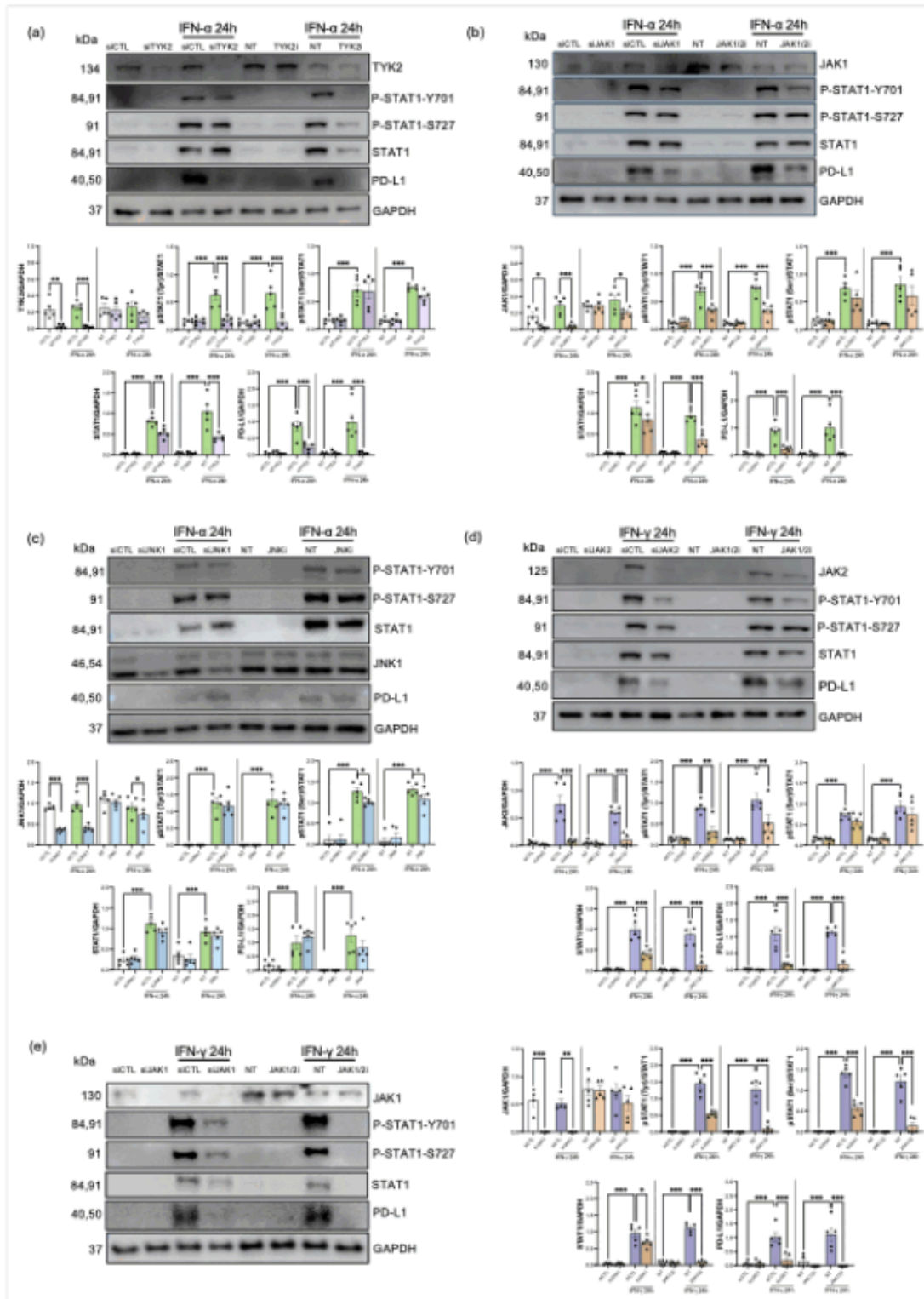


Figure 8

Differential regulation of STAT1 expression and phosphorylation downstream of IFN- α and IFN- γ signaling in EndoC- β H1 cells.

EndoC- β H1 cells were transfected with siRNA control (siCTL) or siRNAs targeting TYK2, JAK1, JAK2 or JNK1. After 24 h of recovery, the cells were left untreated or treated with IFN- α (2,000 U/mL) or IFN- γ (1,000 U/mL) for 24 h. In parallel, cells were pretreated for 2 h with the selective TYK2 inhibitor deucravacitinib (0.3 μ mol/L), the JAK1/2 inhibitor baricitinib (4 μ mol/L), or the JNK inhibitor SP600125 (20 μ mol/L) and then left untreated or treated with IFN- α (2,000 U/mL) or IFN- γ (1,000 U/mL) for 24 h, in the presence of the inhibitors. TYK2 (a), JAK1 (b,e), JNK1 (c), and JAK2 (d), P-STAT1-S727, P-STAT1-Y701, STAT1, PD-L1 and GAPDH protein levels were analyzed by immunoblot. Bands were quantified by densitometry and corrected by GAPDH for TYK2, JAK1, JNK1, JAK2, JNK1, PD-L1 and STAT1; and corrected by STAT1 for P-STAT1-S727 and P-STAT1-Y701, and representative western blots are shown. The results are mean $\pm$ SEM of 5 independent experiments. * P < 0.05, ** P < 0.01, and *** P < 0.01. One-way ANOVA followed by Bonferroni correction for multiple comparisons post-hoc test.

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