Deucravacitinib, a tyrosine kinase 2 pseudokinase inhibitor, protects human beta cells against proinflammatory insults

Reinaldo Sousa Dos Santos[1,2*], Daniel Guzman Llorens[1,2], Atenea Alexandra Perez-Serna[1,2], Angel Nadal[1,2], Laura Marroqui[1,2*]

[1]Instituto de Investigación, Desarrollo e Innovación en Biotecnología Sanitaria de Elche (IDiBE), Universidad Miguel Hernández de Elche, Alicante, Spain.

[2]CIBER de Diabetes y Enfermedades Metabólicas Asociadas, Instituto de Salud Carlos III.

*** Corresponding authors:**

Reinaldo Sousa Dos Santos

Instituto de Investigación, Desarrollo e Innovación en Biotecnología Sanitaria de Elche

Universidad Miguel Hernández de Elche, Ed. Torregaitán, Av. de la Universidad s/n 03202 Elche, Spain.

Email: r.sousa@umh.es

Laura Marroqui

Instituto de Investigación, Desarrollo e Innovación en Biotecnología Sanitaria de Elche

Universidad Miguel Hernández de Elche, Ed. Torregaitán, Av. de la Universidad s/n 03202 Elche, Spain.

Email: lmarroqui@umh.es

Abstract

Aims/hypothesis: Type 1 diabetes is characterised by pancreatic islet inflammation and autoimmune-driven pancreatic beta cell destruction. Type I interferons, such as IFNα, are key players in early human type 1 diabetes pathogenesis, as the activation of the tyrosine kinase 2 (TYK2)-signal transducer and activator of transcription (STAT) pathway induces inflammation, a long-lasting MHC class I overexpression, endoplasmic reticulum (ER) stress, and beta cell apoptosis (in synergy with IL-1β). As TYK2 inhibition has been suggested as a potential therapeutic target for the prevention or treatment of type 1 diabetes, we investigated whether the selective TYK2 inhibitor deucravacitinib could protect beta cells against the damaging effects of IFNα and other proinflammatory cytokines (i.e. IFNγ and IL-1β).

Methods: Inflammation, ER stress, and apoptosis were evaluated by real-time PCR, immunoblot, immunofluorescence, and nuclear dyes. The promoter activity was assessed by luciferase assay and insulin secretion and content by ELISA. All experiments were performed in the human EndoC-βH1 cell line.

Results: Pre-treatment with deucravacitinib prevented IFNα effects, such as STAT1 and STAT2 phosphorylation and protein expression as well as MHC class I hyperexpression, in a dose-dependent manner without affecting beta cell survival and function. Comparison between deucravacitinib and two Janus kinase inhibitors, ruxolitinib and baricitinib, showed that deucravacitinib blocked IFNα- but not IFNγ-induced signalling pathway. Pre-treatment with deucravacitinib protected beta cells from the pro-apoptotic and proinflammatory effects of two different combinations of cytokines: IFNα + IL-1β and IFNγ + IL-1β. Moreover, this TYK2 inhibitor could partially revert apoptosis and inflammation in cells previously treated with IFNα + IL-1β or IFNγ + IL-1β.

Conclusions/interpretation: Our findings suggest that, by protecting beta cells against the deleterious effects of proinflammatory cytokines without affecting beta cell function and survival, deucravacitinib could be repurposed for the prevention or treatment of early type 1 diabetes.

Keywords: Apoptosis, Deucravacitinib, Inflammation, Pancreatic beta cells, TYK2, Type 1 diabetes, Type I interferons

Introduction

Type 1 diabetes is characterised by pancreatic islet inflammation and specific destruction of pancreatic beta cells by an autoimmune assault, which develops in the context of an inadequate "dialogue" between beta cells and the invading immune cells [1, 2].

A growing body of evidence places type I interferons (IFNs) as key players in the early stages of human type 1 diabetes pathogenesis [3]. IFNα was found in islets from type 1 diabetes patients [4–6], and laser-captured islets from living donors with recent-onset type 1 diabetes showed increased expression of IFN-stimulated genes (ISGs) [7]. In genetically susceptible children, an IFN signature was temporarily amplified preceding the development of autoantibodies and throughout the progress of type 1 diabetes [8, 9]. Recently, three type I IFN response markers, namely human MX Dynamin Like GTPase 1 (MX1), double-stranded RNA sensor protein kinase R, and HLA class I, were found to be expressed in a significantly higher percentage of insulin-containing islets from autoantibody-positive and/or recent-onset type 1 diabetes donors [10]. In human beta cells, IFNα induces inflammation, endoplasmic reticulum (ER) stress as well as a long-lasting overexpression of HLA class I via activation of the tyrosine kinase 2 (TYK2)-signal transducer and activator of transcription (STAT) pathway. Moreover, IFNα induces apoptosis in the presence of IL-1β [11–14].

Targeting the type I IFN signalling pathway has been proposed as a potential adjuvant therapy to treat at-risk individuals or patients still in the very early stages of the disease [3, 15]. Among some of the strategies that have been suggested, inhibitors of Janus kinase (JAK) proteins (JAK1-3 and TYK2) show great promise. Treatment with AZD1480 (a JAK1/JAK2 inhibitor) and ABT 317 (a JAK1-selective inhibitor) protected non-obese diabetic mice against autoimmune diabetes and reversed diabetes in newly diagnosed non-diabetic mice [16, 17]. In human beta cells, clinically used JAK inhibitors, namely ruxolitinib, cerdulatinib, and baricitinib, prevented MHC class I overexpression, ER stress, chemokine production, and apoptosis [13, 14].

Lately, attention has been focused on *TYK2*, a candidate gene for type 1 diabetes whose genetic variants that decrease TYK2 activity are associated with protection against the disease [18–20]. TYK2 is crucial for cell development and IFNα-mediated responses in human beta cells [11, 21, 22]. Partial TYK2 knockdown protected human beta cells against apoptosis and inflammation induced by polyinosinic-polycitidilic acid (poly(I:C)), a mimic of double-stranded RNA produced during viral infection [21]. In mature stem cell-islets, TYK2 knockout or pharmacological

83 inhibition decreased T-cell-mediated cytotoxicity by preventing IFNα-induced antigen processing
84 and presentation, including MHC class I expression [22]. As these findings place TYK2 as a
85 critical regulator of the type I IFN signalling pathway in beta cells, selective TYK2 inhibition has
86 emerged as a drug target to treat type 1 diabetes. Recently, two novel small molecule inhibitors
87 binding to the TYK2 pseudokinase domain protected human beta cells against the deleterious
88 effects of IFNα without compromising beta cell function and susceptibility to potentially
89 diabetogenic viruses [23].
90 Deucravacitinib (BMS-986165), a small molecule that selectively targets the TYK2 pseudokinase
91 domain, has shown great therapeutic potential for immune-mediated diseases, such as lupus
92 nephritis and systemic lupus erythematosus [24, 25]. In fact, deucravacitinib has been recently
93 approved for treatment of plaque psoriasis [26–28]. However, no preclinical studies have deeply
94 explored the possible use of deucravacitinib in the context of type 1 diabetes. Notably, Chandra et
95 al. recently used deucravacitinib to validate their CRISPR-Cas9-generated *TYK2* knockout in
96 human induced pluripotent stem cells, but did not provide further characterisation of its effects in
97 beta cells [22].
98 In the present study, we described the effects of deucravacitinib on the human EndoC-βH1 beta
99 cell line, including its ability to prevent IFNα-triggered signalling pathway and subsequent
100 damaging effects on beta cells. We report that deucravacitinib prevented IFNα effects in a dose-
101 dependent manner without affecting beta cell survival and function. Compared with ruxolitinib
102 and baricitinib, deucravacitinib inhibited the IFNα- but not IFNγ-stimulated signalling pathway.
103 Interestingly, this TYK2 inhibitor protected beta cells not only against the deleterious effects of
104 IFNα but also from other proinflammatory cytokines, namely IFNγ and IL-1β, which suggests that
105 deucravacitinib could be introduced as an adjuvant protective therapy at different stages of the
106 disease to avoid the progressive loss of beta cell mass.
107
108 **Methods**
109 **Culture of EndoC-βH1 cells**
110 The human EndoC-βH1 beta cell line [research resource identifier (RRID): CVCL_L909,
111 Univercell-Biosolutions, France] was cultured in Matrigel/fibronectin-coated plates as previously
112 described [29]. Cells were cultured in DMEM containing 5.6 mmol/l glucose, 10 mmol/l
113 nicotinamide, 5.5 μg/ml transferrin, 50 μmol/l 2-mercaptoethanol, 6.7 ng/ml selenite, 2% BSA

114 fatty acid free, 100 U/ml penicillin, and 100 µg/ml streptomycin. We confirmed that cells were
115 mycoplasma-free using the MycoAlert Mycoplasma Detection Kit (Lonza, Basel, Switzerland).
116
117 **Cell treatments**
118 Proinflammatory cytokine concentrations were selected according to previously established
119 experiments in human beta cells [11, 30]: recombinant human IFNα (PeproTech Inc., Rocky Hill,
120 NJ) at 1000 U/ml; recombinant human IFNγ (PeproTech Inc., Rocky Hill, NJ) at 1000 U/ml; and
121 recombinant human IL-1β (R&D Systems, Abingdon, UK) at 50 U/ml. Cells were transfected with
122 1 µg/ml poly(I:C) (InvivoGen, San Diego, CA) as indicated [31]. Ruxolitinib, baricitinib, or
123 deucravacitinib (Selleckchem, Planegg, Germany) were prepared by dissolution in DMSO (used
124 as vehicle) and cells were treated as indicated in the figures. Ruxolitinib and baricitinib
125 concentrations were selected based on previous dose-response experiments (unpublished data).
126 For treatments involving cytokines, 2% FBS was added to the culture medium.
127
128 **Cell viability assessment**
129 The percentage of apoptosis was measured by fluorescence microscopy upon staining with the
130 DNA-binding dyes Hoechst 33342 and propidium iodide (Sigma-Aldrich, Saint Louis, MO, USA)
131 as described [32]. At least 600 cells were counted for each experimental condition. Viability was
132 assessed by two independent observers, one of whom was unaware of sample identity, with an
133 agreement between results of >90%.
134
135 **Caspase 3/7 activity**
136 Caspase 3/7 activity was determined using the Caspase-Glo® 3/7 assay (Promega, Madison, WI,
137 USA) following the manufacturer's instructions. Briefly, upon treatment in 100 µl culture medium,
138 cells were incubated with 100 µl Caspase-Glo® 3/7 reagent at room temperature for 1 h before
139 recording luminescence with a POLASTAR plate reader (BMG Labtech, Ortenberg, Germany).
140
141 **C-X-C motif chemokine ligand 10 (CXCL10) measurements**
142 The release of CXCL10 to the culture medium was detected using Human ProcartaPlex
143 immunoassays (Invitrogen, Vienna, Austria) following the manufacturer's recommendations.
144 Reactions were read with a MagPix system (Luminex, Austin, TX, USA).

145

146 **Luciferase reporter assays**

147 EndoC-βH1 cells were transfected using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA)
148 with pRL-CMV encoding *Renilla* luciferase (Promega) and luciferase reporter constructs for either
149 gamma-interferon activation site (GAS) (Panomics, Fremont, CA, USA) or IFN-stimulated
150 regulatory element (ISRE) (kindly provided by Dr Izortze Santin, University of the Basque
151 Country, Spain). After recovery, cells were treated with either IFNα for 2 h or IFNγ for 24 h [33].
152 Luciferase activity was measured in a POLASTAR plate reader (BMG Labtech) using the Dual-
153 Luciferase Reporter Assay System (Promega) and corrected for the luciferase activity of the
154 internal control plasmid, i.e. pRL-CMV.

155

156 **RNA analysis**

157 Poly(A)$^+$ mRNA was extracted using Dynabeads mRNA DIRECT kit (Invitrogen) and cDNA
158 synthesis was performed using the High-Capacity cDNA Reverse Transcription Kit (Applied
159 Biosystems). Real-time PCR was performed on the CFX96 Real Time System (Bio-Rad) as
160 described [34]. The housekeeping gene β-actin was used to correct expression values ($2^{-\Delta Ct}$) [35].
161 All primers used here are listed in ESM Table 1.

162

163 **Immunoblotting and immunofluorescence analyses**

164 Western blotting analysis was performed as described [21]. Briefly, cells were washed with cold
165 PBS and lysed in Laemmli buffer. Immunoblot was performed using antibodies against STAT1
166 and STAT2 (phosphorylated and total forms; all at 1:1000 dilution), and α-tubulin (1:5000).
167 Peroxidase-conjugated antibodies (1:5000) were used as secondary antibodies. SuperSignal West
168 Femto chemiluminescent substrate (Thermo Scientific, Rockford, IL, USA) and ChemiDoc XRS+
169 (Bio-Rad Laboratories, Hercules, CA, USA) were used to detect bands.

170 Immunofluorescence was carried out as described [11, 21]. First, cells were washed with cold PBS
171 and fixed with 4% paraformaldehyde. Afterwards, cells were permeabilised and incubated with
172 the mouse anti-MHC Class I (W6/32) antibody (1:1000). The Alexa Fluor 568 polyclonal goat
173 anti-mouse IgG (1:500) was used as secondary antibody. Upon staining with Hoechst 33342,
174 coverslips were mounted with fluorescent mounting medium (Dako, Carpintera, CA, USA) and
175 immunofluorescence was observed with an inverted fluorescence microscope Zeiss Confocal

176 LSM900 with Airyscan 2 microscope equipped with a camera (Zeiss-Vision, Munich, Germany),
177 and images were acquired at x40 magnification and analysed using ZEN software (version 3.3;
178 Zeiss-Vision, Munich, Germany) and open-source FIJI software (version 2.0; https://fiji.sc).
179 All antibodies used here are provided in ESM Table 2.
180
181 **Glucose-stimulated insulin secretion**
182 After preincubation in modified Krebs-Ringer for 1 h, cells were sequentially stimulated with low
183 (0 mmol/l) and high glucose (20 mmol/l) for 1 h (each stimulation) as previously described [36].
184 Insulin secreted and insulin content from lysed cells were measured using a human insulin ELISA
185 kit (Mercodia, Uppsala, Sweden) following the manufacturer's instructions. See ESM Methods for
186 further details.
187
188 **Statistical analyses**
189 The GraphPad Prism 7.0 software (GraphPad Software, La Jolla, CA, USA) was used for statistical
190 analyses. Data are shown as mean ± SEM of independent experiments (i.e. considering EndoC-
191 βH1 cells from different passages as $n = 1$, with individual data added to the column bars). The
192 statistical significance of differences between groups was evaluated using one-way ANOVA
193 followed by Dunnett's test or two-way ANOVA followed by Sidak's test or by Dunnett's test, as
194 appropriate.
195
196 **Results**
197 **Deucravacitinib prevents IFNα effects in EndoC-βH1 cells**
198 IFNα-mediated JAK/TYK2 activation leads to phosphorylation of STAT1 and STAT2, which will
199 eventually upregulate several ISGs, including STATs, *HLA-ABC*, *CXCL10*, and *MX1* (ESM Fig.
200 1a). Pre-treatment of EndoC-βH1 cells with deucravacitinib inhibited IFNα-induced STAT1 and
201 STAT2 phosphorylation in a dose-dependent manner, where deucravacitinib showed greater
202 potency towards IFNα-stimulated STAT1 inhibition (Fig. 1a,b). We then selected two doses, 10
203 and 1000 nmol/l, for the follow-up experiments. Next, we examined the effect of deucravacitinib
204 on the kinetics of IFNα-induced STAT activation. As expected, the phosphorylation of STAT1
205 and STAT2 was markedly amplified by IFNα at early time points (1–4 h) and returned to baseline
206 by 24 h (Fig. 1c,d and ESM Fig. 1b). STAT1 and STAT2 protein expression augmented in a time-

207 dependent manner. Although both proteins were already upregulated by 8 h, STAT2 reached an
208 expression peak at 16 h, while STAT1 expression was still increasing by 24 h (Fig. 1c-f). Exposure
209 to 1000 nmol/l deucravacitinib abrogated the IFNα-stimulated STAT1 and STAT2
210 phosphorylation and protein expression, whereas 10 nmol/l deucravacitinib had only a minor effect
211 (Fig. 1c-f and ESM Fig. 1b). These findings are better evidenced by analysing the area under the
212 curve of the phosphorylated and total forms of STAT1 and STAT2 (ESM Fig. 1c-f). Finally, MHC
213 class I protein expression stimulated by IFNα- was completely blocked by 1000 nmol/l
214 deucravacitinib (Fig. 1g,h).
215
216 **Beta cell survival and function are not affected by deucravacitinib**
217 We examined whether deucravacitinib would interfere with beta cell survival and function in the
218 absence or presence of IFNα. After 24 h exposure, 1000 nmol/l deucravacitinib did not affect beta
219 cell viability (ESM Fig. 2a) nor change glucose-stimulated insulin secretion and insulin content
220 (ESM Fig. 2b,c). IFNα did not affect beta cell viability and function as described [11, 23].
221
222 **IFNα, but not IFNγ signalling pathway is blocked by deucravacitinib**
223 We compared deucravacitinib with ruxolitinib and baricitinib, two JAK1/JAK2 inhibitors
224 previously tested in beta cells [13, 14, 37]. First, we measured STAT1 and STAT2 phosphorylation
225 upon stimulation with IFNα or IFNγ (Fig. 2a-d and ESM Fig. 3a,b). Ruxolitinib, baricitinib, and
226 deucravacitinib prevented IFNα-stimulated increase in P-STAT1 and P-STAT2 levels (Fig. 2a,c
227 and ESM Fig. 3b). Nevertheless, deucravacitinib did not change IFNγ-induced STAT1
228 phosphorylation, whereas ruxolitinib and baricitinib blocked it (Fig. 2b,d). Of note, IFNγ did not
229 induce STAT2 phosphorylation (Fig. 2b) [33]. Next, we assessed ISRE and GAS activities upon
230 stimulation with IFNα and IFNγ (Fig. 2e,f and ESM Fig. 3c,d). All three inhibitors abrogated
231 IFNα-stimulated ISRE reporter activity (Fig. 2e), whereas IFNγ-induced GAS activation was
232 barely affected by deucravacitinib (Fig. 2f). As expected, ISRE and GAS activities were not
233 stimulated by, respectively, IFNγ and IFNα (ESM Fig. 3c,d). These findings corroborate the
234 deucravacitinib mode of action, which specifically binds to and inhibits TYK2 without affecting
235 JAK1/JAK2 pathways. As TYK2 does not participate in the IFNγ pathway, the lack of
236 deucravacitinib effect in IFNγ-stimulated changes is expected.
237

238 **Deucravacitinib blocks IFNα-induced upregulation of ISGs, but not ER stress markers**
239 We assessed the effects of these three inhibitors on the expression of some ISGs and ER stress
240 markers. All three inhibitors prevented IFNα-induced upregulation of *HLA-ABC*, *CXCL10*, and
241 *MX1* in a dose-dependent manner (Fig. 3a-c). Although ruxolitinib and baricitinib inhibited the
242 expression of the ER stress markers C/EBP homologous protein (*CHOP*, also known as *DDIT3*)
243 and spliced isoform of XBP1 X-box binding protein 1 (*XBP1s*), only the lower dose of
244 deucravacitinib reduced CHOP expression (Fig. 3d,e). None of the three inhibitors changed the
245 mRNA expression of activating transcription factor 3 (*ATF3*) (Fig. 3f).
246
247 **Deucravacitinib prevents IFNα + IL-1β-induced effects in beta cells**
248 Previous studies showed that a combination of IFNα + IL-1β, two cytokines that might be present
249 in the islet milieu at early stages of insulitis, induce beta cell apoptosis, inflammation, and ER
250 stress [11, 14, 23] (Fig. 4a). Then, we investigated whether deucravacitinib protects beta cells after
251 IFNα + IL-1β exposure for 24 h. We observed that deucravacitinib completely prevented IFNα +
252 IL-1β-induced apoptosis, which was assessed by two approaches, namely DNA-binding dyes and
253 caspase 3/7 activity assay (Fig. 4b,c). Moreover, cells treated with deucravacitinib showed reduced
254 levels of P-STAT1 and STAT1 (Fig. 4d-f) as well as *HLA-ABC*, *MX1*, *CXCL10*, and *CHOP* mRNA
255 expression (Fig. 4g-j). MHC class I protein expression and CXCL10 secretion to the medium were
256 also decreased by TYK2 inhibition (Fig. 4k-m).
257
258 **Deucravacitinib abrogates IFNγ + IL-1β-induced apoptosis in beta cells**
259 We then evaluated whether deucravacitinib would protect against cytokines that, as compared with
260 IFNα, probably appear later in the progression of islet inflammation, such as IFNγ and IL-1β [1,
261 38] (Fig. 5a). After treatment for 24 h, deucravacitinib inhibited IFNγ + IL-1β-induced apoptosis
262 in a dose-dependent manner (60% and 92% protection at 10 and 1000 nmol/l, respectively) (Fig.
263 5b). These results were confirmed by the caspase 3/7 activity assay (Fig. 5c). Pre-treatment with
264 deucravacitinib did not block IFNγ + IL-1β-induced STAT1 phosphorylation and protein
265 expression (Fig. 5d-f) or *HLA-ABC* mRNA expression (Fig. 5g). Conversely, deucravacitinib
266 diminished *MX1* and *CXCL10* mRNA expression, whereas *CHOP* was reduced at the lower dose
267 of deucravacitinib (Fig. 5h-j).
268

269 **Poly(I:C)-induced apoptosis is not changed by TYK2 inhibition**

270 As intracellular exposure to poly(I:C), a mimic of viral infection, results in the production and
271 secretion of type I IFNs as well as beta cell apoptosis [21, 39, 40], we tested deucravacitinib after
272 treatment with poly(I:C) for 24 h. Contrary to what we observed for cytokine-triggered cell death
273 (Figs 4 and 5), apoptosis induced by poly(I:C) was not altered by deucravacitinib (ESM Fig. 4a).
274 Curiously, despite the absence of an effect on viability, deucravacitinib inhibited poly(I:C)-
275 stimulated *HLA-ABC*, *MX1*, and *CHOP* upregulation (ESM Fig. 4b-d). While *CXCL10* mRNA
276 expression was dampened by TYK2 inhibition, CXCL10 protein secretion was not significantly
277 reduced (ESM Fig. 4e,f).

278

279 **The harmful effects of cytokines are partially reverted by deucravacitinib**

280 Up to this point, we investigated whether pre-treatment with deucravacitinib would prevent the
281 effects of different cytokines in beta cells. Here, we assessed if deucravacitinib could reverse these
282 damaging effects. EndoC-βH1 cells were pre-treated with either IFNα + IL-1β or IFNγ + IL-1β
283 for 24 h. Afterwards, 1000 nmol/l deucravacitinib was added for an additional 24 h still in the
284 presence of the different mixes of cytokines (Figs 6a and 7a). IFNα + IL-1β-induced apoptosis was
285 partially reversed by deucravacitinib (60% decrease) (Fig. 6b). The expression of *HLA-ABC*
286 mRNA stimulated by IFNα + IL-1β remained unchanged in the presence of deucravacitinib (Fig.
287 6c), which agrees with previous data showing a long-lasting expression of *HLA-ABC* [13]. STAT1
288 protein levels and CXCL10 secretion as well as *CHOP* mRNA expression were reduced by 26%-
289 42%, while the expression of *MX1* and *CXCL10* was completely inhibited by deucravacitinib (Fig.
290 6 d-i). Of note, STAT1 phosphorylation was not detected, probably due to a process of
291 desensitisation following the initial IFNα stimulus [33] (Fig. 6c).

292 Similarly to IFNα + IL-1β (Fig. 6), deucravacitinib diminished IFNγ + IL-1β-induced apoptosis
293 (64% decrease) but did not modify *HLA-ABC* mRNA expression (Fig. 7b,c). Protein levels of
294 STAT1 and CXCL10, however, were not altered by the TYK2 inhibitor, whereas a slight, non-
295 significant 30% reduction was seen in *CHOP* expression (Fig. 7d,e,g,i). Expression of *MX1* and
296 *CXCL10* was only partially blocked by deucravacitinib under IFNγ + IL-1β conditions (Fig. 7f,h).

297

298 **Discussion**

299 Due to its implication in type 1 diabetes pathogenesis, IFNα pathway has arisen as an interesting
300 therapeutic target. Reduction of IFNα extracellular levels, blockade of IFNα itself and/or its
301 receptor, and reduction of the activity of proteins mediating IFN effects have been proposed as
302 means to diminish IFNα deleterious effects [41]. In rodent models, some of these strategies have
303 successfully prevented diabetes development [16, 17, 42–44].
304 One of the most promising therapeutic approaches for type 1 diabetes prevention/early treatment
305 is the targeting of the JAK-STAT pathway with JAK inhibitors [3, 15]. This strategy has been
306 clinically approved for the treatment of some autoimmune diseases, including systemic lupus
307 erythematosus [45], rheumatoid arthritis [46], and psoriasis [26, 27]. Although there are no
308 approved JAK inhibitors being clinically used for type 1 diabetes, recent preclinical data suggest
309 that these inhibitors could be repurposed to treat this disease [13, 14, 16, 17, 22, 23]. In fact, a
310 clinical trial aiming to determine whether baricitinib could slow the progressive, immune-
311 mediated loss of beta cell function and mass that occurs in type 1 diabetes is currently ongoing
312 [47].
313 In the current study, we tested whether the TYK2 inhibitor deucravacitinib could protect human
314 beta cells against the deleterious effects of IFNα and other cytokines. We chose to focus on this
315 TYK2 inhibitor for two main reasons: first, due to the importance of TYK2 to type 1 diabetes
316 pathogenesis. For instance, TYK2 regulates pro-apoptotic and proinflammatory pathways via
317 regulation of the IFNα signalling, antigen processing and presentation, and modulation of cytokine
318 and chemokine production in beta cells [21, 22]. Second, exploring a drug that has been recently
319 approved by the US Food and Drug Administration to treat another autoimmune disease, namely
320 plaque psoriasis [28], increases its repositioning potential for type 1 diabetes and facilitates the
321 bench-to-bedside transition.
322 Deucravacitinib is a small-molecule ligand that binds to and stabilizes the TYK2 pseudokinase
323 domain, leading to highly potent and selective allosteric TYK2 inhibition [24, 48]. Inhibition of
324 IFNα-induced STAT phosphorylation by deucravacitinib has been shown in several cell types,
325 such as $CD3^+$ T cells, $CD19^+$ B cells, and $CD14^+$ monocytes [24]. Here we showed that
326 deucravacitinib also prevents IFNα-stimulated STAT1 and STAT2 phosphorylation in human
327 EndoC-βH1 cell line. Furthermore, in agreement with previous findings [24], deucravacitinib also
328 showed higher potency against TYK2-mediated phosphorylation of STAT1 compared with
329 STAT2 phosphorylation in our experimental model. Notably, at the concentrations used in our

330 study, deucravacitinib did not affect beta cell function and viability, which is a desired feature for
331 a drug with therapeutic potential.
332 Compared with ruxolitinib and baricitinib, two clinically available JAK1/JAK2 inhibitors,
333 deucravacitinib was more potent against IFNα-stimulated STAT phosphorylation, ISRE activity,
334 and mRNA expression of *HLA-ABC*, *MX1*, and *CXCL10*. However, unlike ruxolitinib and
335 baricitinib, deucravacitinib did not block the IFNα-mediated upregulation of the ER stress markers
336 *CHOP* and *XBP1s*. Our results partially agree with the ones reported by Coomans de Brachène et
337 al., where two TYK2 inhibitors failed to prevent IFNα-induced *CHOP* expression in EndoC-βH1
338 cells but inhibited *CHOP* and *ATF3* expression in dispersed human islets [23].
339 Prior studies have shown that other JAK/TYK2 inhibitors could prevent the detrimental effects of
340 IFNα + IL-1β, such as apoptosis and inflammation [14, 23]. Therefore, we proceeded to
341 investigate whether deucravacitinib could protect beta cells against the harmful effects of two
342 different combinations of cytokines: IFNα + IL-1β (early insulitis) and IFNγ + IL-1β (late
343 insulitis). In both scenarios, pre-treatment with deucravacitinib not only protected against
344 cytokine-induced apoptosis but prevented the upregulation of *HLA-ABC*, *MX1*, *CXCL10*, and
345 *CHOP*. Additionally, in cells treated with IFNα + IL-1β, deucravacitinib blocked the
346 overexpression of MHC class I at the cell surface and CXCL10 secretion to the medium.
347 Importantly, the addition of deucravacitinib when cytokine exposure was already ongoing could
348 reverse, at least in part, the deleterious effects of these cytokines. Although it seems clear that
349 deucravacitinib confers protection against IFNα + IL-1β by directly inhibiting the TYK2-mediated
350 pathway, it remains to be answered how deucravacitinib protects against IFNγ + IL-1β-induced
351 effects. Indeed, our present data suggest that deucravacitinib does not interfere with the IFNγ-
352 mediated signalling pathway.
353 Due to TYK2 role in the IFNα-mediated antiviral response, as evidenced by the regulation of *MX1*
354 mRNA expression and STAT1 protein levels [11, 22, 23], another desired characteristic for a
355 TYK2 inhibitor would be to block IFNα pathway without sensitising beta cells to viral infections.
356 To test this hypothesis, we mimicked a viral infection by exposing EndoC-βH1 cells to poly(I:C).
357 In our model, deucravacitinib abrogated poly(I:C)-stimulated inflammation but did not change
358 poly(I:C)-induced beta cell apoptosis. Even though our results were obtained with a synthetic viral
359 double-stranded RNA analogue, they conform to a previous study showing that two novel TYK2
360 inhibitors did not sensitize human beta cells to potentially diabetogenic coxsackieviruses CVB1

361 and CVB5 [23]. Importantly, deucravacitinib and these two TYK2 inhibitors share the same
362 mechanism of action, i.e. binding to the TYK2 pseudokinase domain.
363 Based on our findings, it will be interesting to test whether novel small molecule TYK2
364 pseudokinase ligands [49] could also protect beta cells from IFNα deleterious effects.
365 Nevertheless, we must bear in mind that completely inhibiting TYK2 may be counterproductive,
366 as it might lead to susceptibility to microorganisms (e.g. mycobacteria and virus) and
367 immunodeficiency [50]. Thus, regardless of the TYK2 inhibitor chosen, we should focus on doses
368 that induce a partial inhibition, as seen in individuals with a protective single nucleotide
369 polymorphism in the *TYK2* gene [18], as it could offer maximal efficacy with reduced risk of
370 developing secondary infections.
371 One potential limitation of our study is its purely in vitro nature, which may limit our conclusions
372 regarding the use of deucravacitinib to treat a disease as complex as type 1 diabetes. Conversely,
373 our findings, along with those reported by Coomans de Brachène et al. [23] and Chandra et al.
374 [22], provide further preclinical evidence that TYK2 inhibitors could be considered a strategy for
375 an early therapy for type 1 diabetes. The next logical step would be to investigate whether our in
376 vitro findings could be translated to animal models of type 1 diabetes (e.g. NOD and RIP-B7.1
377 mice).
378 In conclusion, we have provided evidence that deucravacitinib protects beta cells against the
379 deleterious effects of proinflammatory cytokines, such as IFNα, IFNγ and IL-1β, without affecting
380 beta cell function and survival. Our present findings add to the growing body of evidence showing
381 that TYK2 inhibition may be an efficient strategy to treat type 1 diabetes. Moreover, we suggest
382 that deucravacitinib could be repurposed to treat pre-symptomatic type 1 diabetes subjects (i.e.
383 positive for 2–3 autoantibodies but still normoglycemic) or be introduced in the early stages of
384 type 1 diabetes onset.
385
386 **Acknowledgements**
387 The authors are grateful to Beatriz Bonmati Botella, Maria Luisa Navarro, and Salomé Ramon for
388 their excellent technical support. Once again, we thank Dr. Izortze Santin, University of the Basque
389 Country, Spain, for providing the luciferase reporter construct for ISRE. Figures 4a, 5a, 6a, 7a,
390 ESM Fig. 1a, and ESM Fig. 3a were created with BioRender.com.
391

Data availability

All data generated or analysed during this study are included in this published article (and its supplementary information files). Data are however available from the corresponding authors upon reasonable request.

Funding

LM is funded by the grant PID2020-117569RA-I00 by MCIN/AEI/10.13039/501100011033 and by the grant SEJI/2018/023 by Generalitat Valenciana. AN is supported by European Union's Horizon 2020 research and innovation programme under grant agreement GOLIATH No. 825489, by the grant PID2020-117294RB-I00 by MCIN/AEI/10.13039/501100011033, and by the grant PROMETEO II/2020/006 by Generalitat Valenciana. This research was supported by CIBER-Consorcio Centro de Investigación Biomédica en Red (CB07/08/0002), Instituto de Salud Carlos III, Ministerio de Ciencia e Innovación.

Authors' relationships and activities

The authors declare that there are no relationships or activities that might bias, or be perceived to bias, their work.

Contribution statement

RSDS and LM: Conceptualization, Supervision, Visualization, Investigation, Formal analysis, and Writing – original draft & editing; DGL and AAP-S: Investigation, Formal analysis, and Writing – review & editing; AN: Resources and Writing – review & editing; LM: Resources, Funding acquisition, and Project administration. All authors have read and given approval to the final version of the manuscript. RSDS and LM are guarantors of this work and, as such, had full access to all the data presented herein and take responsibility for the integrity of the data and the accuracy of data analyses.

References

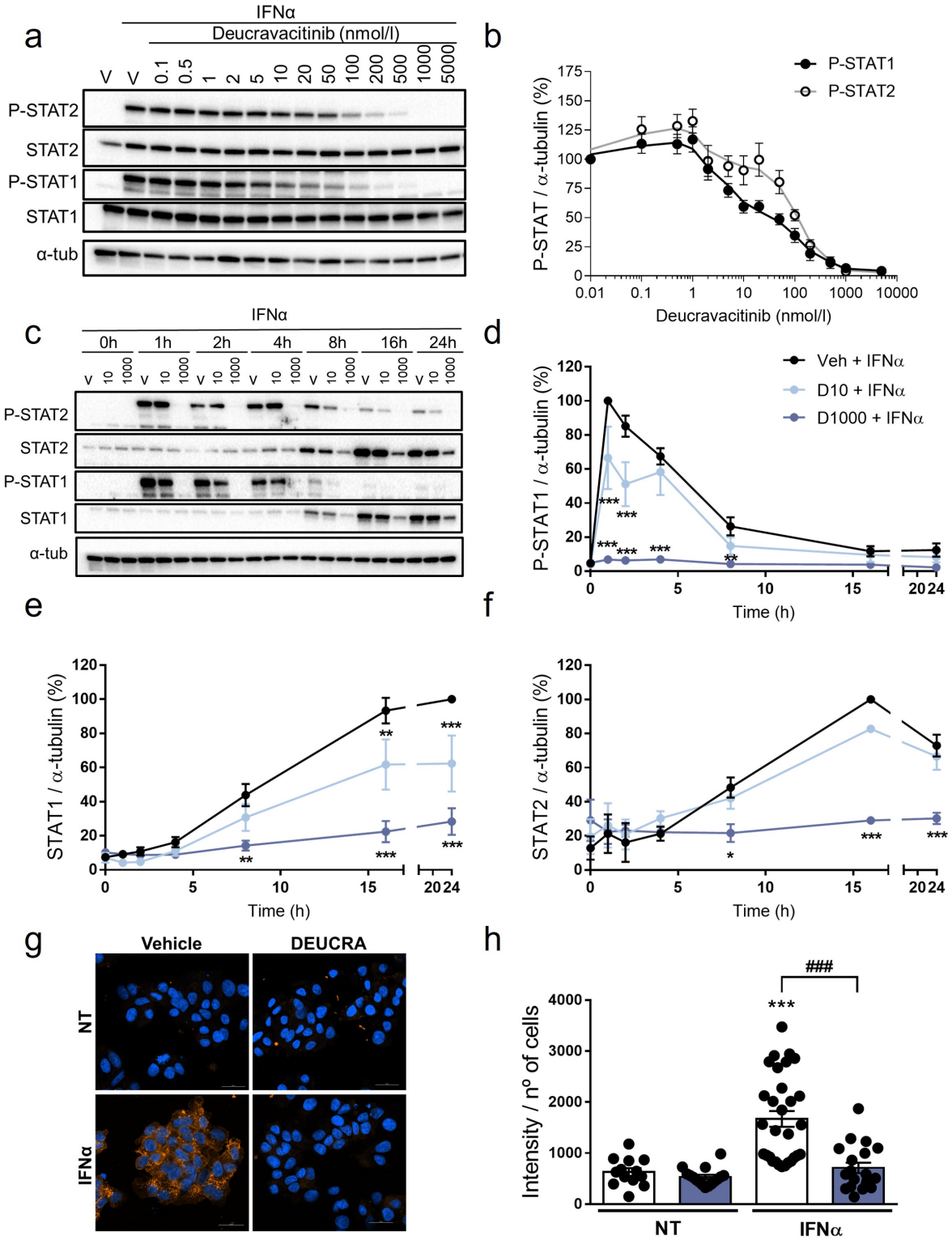
1. Eizirik DL, Pasquali L, Cnop M (2020) Pancreatic β-cells in type 1 and type 2 diabetes mellitus: different pathways to failure. Nat Rev Endocrinol 26(7):349–362. https://doi.org/10.1038/s41574-020-0355-7
2. Mallone R, Eizirik DL (2020) Presumption of innocence for beta cells: why are they vulnerable autoimmune targets in type 1 diabetes? Diabetologia 63(10):1999–2006.

https://doi.org/10.1007/s00125-020-05176-7
3. Marroqui L, Perez-Serna AA, Babiloni-Chust I, Dos Santos RS (2021) Type I interferons as key players in pancreatic β-cell dysfunction in type 1 diabetes. Int Rev Cell Mol Biol 359:1–80. https://doi.org/10.1016/bs.ircmb.2021.02.011
4. Foulis A, Farquharson M, Meager A (1987) Immunoreactive alpha-interferon in insulin-secreting beta cells in type 1 diabetes mellitus. The Lancet 2(8573):1423–1427. https://doi.org/10.1016/S0140-6736(87)91128-7
5. Somoza N, Vargas F, Roura-Mir C, et al (1994) Pancreas in recent onset insulin-dependent diabetes mellitus: Changes in HLA, adhesion molecules and autoantigens, restricted T cell receptor Vβ usage, and cytokine profile. J Immunol 153(3):1360–1377
6. Huang X, Yuan J, Goddard A, et al (1995) Interferon expression in the pancreases of patients with type I diabetes. Diabetes 44(6):658–664. https://doi.org/10.2337/diab.44.6.658
7. Lundberg M, Krogvold L, Kuric E, Dahl-Jørgensen K, Skog O (2016) Expression of interferon-stimulated genes in insulitic pancreatic islets of patients recently diagnosed with type 1 diabetes. Diabetes 65(10):3104–3110. https://doi.org/10.2337/db16-0616
8. Ferreira RC, Guo H, Coulson RMR, et al (2014) A type I Interferon transcriptional signature precedes autoimmunity in children genetically at risk for type 1 diabetes. Diabetes 63(7):2538–2550. https://doi.org/10.2337/db13-1777
9. Kallionpaa H, Elo LL, Laajala E, et al (2014) Innate Immune Activity Is Detected Prior to Seroconversion in Children With HLA-Conferred Type 1 Diabetes Susceptibility. Diabetes 63(7):2402–2414. https://doi.org/10.2337/db13-1775
10. Apaolaza PS, Balcacean D, Zapardiel-Gonzalo J, et al (2021) Islet expression of type I interferon response sensors is associated with immune infiltration and viral infection in type 1 diabetes. Sci Adv 7(9):eabd6527. https://doi.org/10.1126/sciadv.abd6527
11. Marroqui L, Dos Santos RS, Op de beeck A, et al (2017) Interferon-α mediates human beta cell HLA class I overexpression, endoplasmic reticulum stress and apoptosis, three hallmarks of early human type 1 diabetes. Diabetologia 60(4):656–667. https://doi.org/10.1007/s00125-016-4201-3
12. Lombardi A, Tomer Y (2017) Interferon alpha impairs insulin production in human beta cells via endoplasmic reticulum stress. J Autoimmun 80:48–55. https://doi.org/10.1016/j.jaut.2017.02.002
13. Coomans de Brachène A, Dos Santos RS, Marroqui L, et al (2018) IFN-α induces a preferential long-lasting expression of MHC class I in human pancreatic beta cells. Diabetologia 61(3):636–640. https://doi.org/10.1007/s00125-017-4536-4
14. Colli ML, Ramos-Rodríguez M, Nakayasu ES, et al (2020) An integrated multi-omics approach identifies the landscape of interferon-α-mediated responses of human pancreatic beta cells. Nat Commun 11(1):2584. https://doi.org/10.1038/s41467-020-16327-0
15. Eizirik DL, Szymczak F, Alvelos MI, Martin F (2021) From Pancreatic β-Cell Gene Networks to Novel Therapies for Type 1 Diabetes. Diabetes 70(9):1915–1925. https://doi.org/10.2337/dbi20-0046
16. Trivedi PM, Graham KL, Scott NA, et al (2017) Repurposed JAK1/JAK2 inhibitor reverses established autoimmune insulitis in NOD mice. Diabetes 66(6):1650–1660. https://doi.org/10.2337/db16-1250
17. Ge T, Jhala G, Fynch S, et al (2020) The JAK1 Selective Inhibitor ABT 317 Blocks Signaling Through Interferon-γ and Common γ Chain Cytokine Receptors to Reverse Autoimmune Diabetes in NOD Mice. Front Immunol 11:588543.

https://doi.org/10.3389/fimmu.2020.588543
18. Dendrou CA, Cortes A, Shipman L, et al (2016) Resolving TYK2 locus genotype-To-phenotype differences in autoimmunity. Sci Transl Med 8(363):363ra149. https://doi.org/10.1126/scitranslmed.aag1974
19. Tao JH, Zou YF, Feng XL, et al (2011) Meta-analysis of TYK2 gene polymorphisms association with susceptibility to autoimmune and inflammatory diseases. Mol Biol Rep 38(7):4663–4672. https://doi.org/10.1007/s11033-010-0601-5
20. Wallace C, Smyth DJ, Maisuria-Armer M, Walker NM, Todd JA, Clayton DG (2010) The imprinted DLK1-MEG3 gene region on chromosome 14q32.2 alters susceptibility to type 1 diabetes. Nat Genet 42(1):68–71. https://doi.org/10.1038/ng.493
21. Marroqui L, Dos Santos RS, Fløyel T, et al (2015) TYK2, a candidate gene for type 1 diabetes, modulates apoptosis and the innate immune response in human pancreatic β-cells. Diabetes 64(11):3808–3817. https://doi.org/10.2337/db15-0362
22. Chandra V, Ibrahim H, Halliez C, et al (2022) The type 1 diabetes gene TYK2 regulates β-cell development and its responses to interferon-α. Nat Commun 13(1):6363. https://doi.org/10.1038/s41467-022-34069-z
23. Coomans de Brachène A, Castela A, Op de Beeck A, et al (2020) Pre-clinical evaluation of TYK2 inhibitors for human beta cell protection in type 1 diabetes. Diabetes Obes Metab 22(10):1827–1836. https://doi.org/10.1111/dom.14104
24. Burke JR, Cheng L, Gillooly KM, et al (2019) Autoimmune pathways in mice and humans are blocked by pharmacological stabilization of the TYK2 pseudokinase domain. Sci Transl Med 11(502):eaaw1736. https://doi.org/10.1126/scitranslmed.aaw1736
25. Morand E, Pike M, Merrill JT, et al Deucravacitinib, a Tyrosine Kinase 2 Inhibitor, in Systemic Lupus Erythematosus: A Phase II, Randomized, Double-Blind, Placebo-Controlled Trial. Arthritis Rheumatol n/a(n/a). https://doi.org/10.1002/art.42391
26. Armstrong AW, Gooderham M, Warren RB, et al (2022) 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 88(1):P29-39. https://doi.org/10.1016/j.jaad.2022.07.002
27. Strober B, Thaçi D, Sofen H, et al (2022) Deucravacitinib versus placebo and apremilast in moderate to severe plaque psoriasis: efficacy and safety results from the 52-week, randomized, double-blinded, phase 3 POETYK PSO-2 trial. J Am Acad Dermatol 88(1):P40-51. https://doi.org/10.1016/j.jaad.2022.08.061
28. Bristol Myers Squibb. U.S. Food and Drug Administration Approves Sotyktu™ (deucravacitinib), Oral Treatment for Adults with Moderate-to-Severe Plaque Psoriasis. https://news.bms.com/news/details/2022/U.S.-Food-and-Drug-Administration-Approves-Sotyktu-deucravacitinib-Oral-Treatment-for-Adults-with-Moderate-to-Severe-Plaque-Psoriasis/default.aspx. Accessed 27 Nov 2022
29. Ravassard P, Hazhouz Y, Pechberty S, et al (2011) A genetically engineered human pancreatic β cell line exhibiting glucose-inducible insulin secretion. J Clin Invest 121(9):3589–97. https://doi.org/10.1172/JCI58447
30. Brozzi F, Nardelli TR, Lopes M, et al (2015) Cytokines induce endoplasmic reticulum stress in human, rat and mouse beta cells via different mechanisms. Diabetologia 58(10):2307–2316. https://doi.org/10.1007/s00125-015-3669-6
31. Santin I, Moore F, Grieco FA, Marchetti P, Brancolini C, Eizirik DL (2012) USP18 is a key regulator of the interferon-driven gene network modulating pancreatic beta cell inflammation

517 and apoptosis. Cell Death Dis 3(11):e49. https://doi.org/10.1038/cddis.2012.158
518 32. Santin I, Dos Santos RS, Eizirik DL (2016) Pancreatic beta cell survival and signaling
519 pathways: Effects of type 1 diabetes-associated genetic variants. In: Methods in Molecular
520 Biology. Humana Press, New York, NY, USA, pp 21–54
521 33. Dhayal S, Leslie KA, Baity M, et al (2022) Temporal regulation of interferon signalling in
522 human EndoC-βH1 cells. J Mol Endocrinol 69(2):299–313. https://doi.org/10.1530/JME-21-0224
523 34. Villar-Pazos S, Martinez-Pinna J, Castellano-Muñoz M, et al (2017) Molecular
524 mechanisms involved in the non-monotonic effect of bisphenol-A on Ca2+ entry in mouse
525 pancreatic β-cells. Sci Rep 7(1):11770. https://doi.org/10.1038/s41598-017-11995-3
526 35. Livak KJ, Schmittgen TD (2001) Analysis of Relative Gene Expression Data Using Real-
527 Time Quantitative PCR and the 2−ΔΔCT Method. Methods 25(4):402–408.
528 https://doi.org/10.1006/meth.2001.1262
529 36. Dos Santos RS, Medina-Gali RM, Babiloni-Chust I, Marroqui L, Nadal A (2022) In Vitro
530 Assays to Identify Metabolism-Disrupting Chemicals with Diabetogenic Activity in a Human
531 Pancreatic β-Cell Model. Int J Mol Sci 23(9):5040. https://doi.org/10.3390/ijms23095040
532 37. Colli ML, Hill JLE, Marroquí L, et al (2018) PDL1 is expressed in the islets of people with
533 type 1 diabetes and is up-regulated by interferons-α and-γ via IRF1 induction. EBioMedicine
534 36:367–375. https://doi.org/10.1016/j.ebiom.2018.09.040
535 38. Colli ML, Szymczak F, Eizirik DL (2020) Molecular Footprints of the Immune Assault on
536 Pancreatic Beta Cells in Type 1 Diabetes. Front Endocrinol 11:666.
537 https://doi.org/10.3389/fendo.2020.568446
538 39. Dogusan Z, Garcia M, Flamez D, et al (2008) Double-stranded RNA induces pancreatic β-
539 cell apoptosis by activation of the toll-like receptor 3 and interferon regulatory factor 3 pathways.
540 Diabetes 57(5):1236–1245. https://doi.org/10.2337/db07-0844
541 40. Dos Santos RS, Marroqui L, Velayos T, et al (2019) DEXI, a candidate gene for type 1
542 diabetes, modulates rat and human pancreatic beta cell inflammation via regulation of the type I
543 IFN/STAT signalling pathway. Diabetologia 62(3):459–472. https://doi.org/10.1007/s00125-018-
544 4782-0
545 41. Stewart TA (2003) Neutralizing interferon alpha as a therapeutic approach to autoimmune
546 diseases. Cytokine Growth Factor Rev 14(2):139–154. https://doi.org/10.1016/S1359-
547 6101(02)00088-6
548 42. Stewart T, Hultgren B, Huang X, Pitts-Meek S, Hully J, MacLachlan N (1993) Induction
549 of type I diabetes by interferon-alpha in transgenic mice. Science 260(5116):1942–1946.
550 https://doi.org/10.1126/science.8100367
551 43. Li Q, Xu B, Michie SA, Rubins KH, Schreriber RD, McDevitt HO (2008) Interferon-α
552 initiates type 1 diabetes in nonobese diabetic mice. Proc Natl Acad Sci U S A 105(34):12439–
553 12444. https://doi.org/10.1073/pnas.0806439105
554 44. Marro BS, Ware BC, Zak J, De La Torre JC, Rosen H, Oldstone MBA (2017) Progression
555 of type 1 diabetes from the prediabetic stage is controlled by interferon-α signaling. Proc Natl
556 Acad Sci U S A 114(14):3708–3713. https://doi.org/10.1073/pnas.1700878114
557 45. Wallace DJ, Furie RA, Tanaka Y, et al (2018) Baricitinib for systemic lupus erythematosus:
558 a double-blind, randomised, placebo-controlled, phase 2 trial. The Lancet 392(10143):222–231.
559 https://doi.org/10.1016/S0140-6736(18)31363-1
560 46. Taylor PC, Keystone EC, Van Der Heijde D, et al (2017) Baricitinib versus Placebo or
561 Adalimumab in Rheumatoid Arthritis. N Engl J Med 376(652–662).
562 https://doi.org/10.1056/NEJMoa1608345

563 47. Waibel M, Thomas HE, Wentworth JM, et al (2022) Investigating the efficacy of baricitinib
564 in new onset type 1 diabetes mellitus (BANDIT)—study protocol for a phase 2, randomized,
565 placebo controlled trial. Trials 23(1):433. https://doi.org/10.1186/s13063-022-06356-z
566 48. Wrobleski ST, Moslin R, Lin S, et al (2019) Highly Selective Inhibition of Tyrosine Kinase
567 2 (TYK2) for the Treatment of Autoimmune Diseases: Discovery of the Allosteric Inhibitor BMS-
568 986165. J Med Chem 62(20):8973–8995. https://doi.org/10.1021/acs.jmedchem.9b00444
569 49. Zhou Y, Li X, Shen R, et al (2022) Novel Small Molecule Tyrosine Kinase 2 Pseudokinase
570 Ligands Block Cytokine-Induced TYK2-Mediated Signaling Pathways. Front Immunol 13
571 50. Minegishi Y, Saito M, Morio T, et al (2006) Human Tyrosine Kinase 2 Deficiency Reveals
572 Its Requisite Roles in Multiple Cytokine Signals Involved in Innate and Acquired Immunity.
573 Immunity 25:745–755. https://doi.org/10.1016/j.immuni.2006.09.009
574
575



576 **Figure 1. Deucravacitinib inhibits IFNα-mediated STAT phosphorylation and MHC class I**
577 **overexpression.** (**a**,**b**) EndoC-βH1 cells were treated with vehicle (V) or pre-treated with the

578 indicated deucravacitinib concentrations for 1 h. Afterwards, cells were left untreated or treated
579 with IFNα (1000 U/ml) in the absence or presence of deucravacitinib for 1 h. (**a**, **b**) Protein
580 expression was measured by western blot. (**a**) Images are representative of four to six independent
581 experiments. (**b**) Densitometry results are shown for P-STAT1 (black circles) and P-STAT2 (white
582 circles). Values were normalised to α-tubulin, and then to the value of IFNα alone of each
583 experiment (considered as 100%). (**c**-**h**) EndoC-βH1 cells were treated with vehicle (V or Veh,
584 black circles) or pre-treated with deucravacitinib (10 [D10, soft blue circles] and 1000 nmol/l
585 [D1000, dark blue circles]) for 1 h. Afterwards, cells were left untreated or treated with IFNα (1000
586 U/ml) in the absence or presence of deucravacitinib for 1–24 h (**c**-**f**) or 24 h (**g**, **h**). (**c**-**f**) Protein
587 expression was measured by western blot. (**c**) Images are representative of three to six independent
588 experiments. (**d**-**f**) Densitometry results are shown for P-STAT1 (**d**), STAT1 (**e**), and STAT2 (**f**).
589 Values were normalised to α-tubulin, and then to the highest value of each experiment (considered
590 as 1). (**g**, **h**) Immunocytochemistry analysis of MHC class I (red) and Hoechst 33342 (blue) upon
591 exposure to IFNα in the absence (white bars) or presence of 1000 nmol/l deucravacitinib (dark
592 blue bars) for 24 h. Representative images of three independent experiments (13–30
593 images/coverslip) (**g**) and quantification (**h**) are shown. Data are means ± SEM of three to six
594 independent experiments. (**d**-**f**) *$p \leq 0.05$, **$p \leq 0.01$ and ***$p \leq 0.001$ vs Vehicle + IFNα (two-way
595 ANOVA plus Dunnett's test). (**h**) ***$p < 0.001$ vs the respective untreated (NT) (two-way ANOVA
596 plus Sidak's test); $^{###}p \leq 0.001$, as indicated by bars (two-way ANOVA plus Dunnett's test).

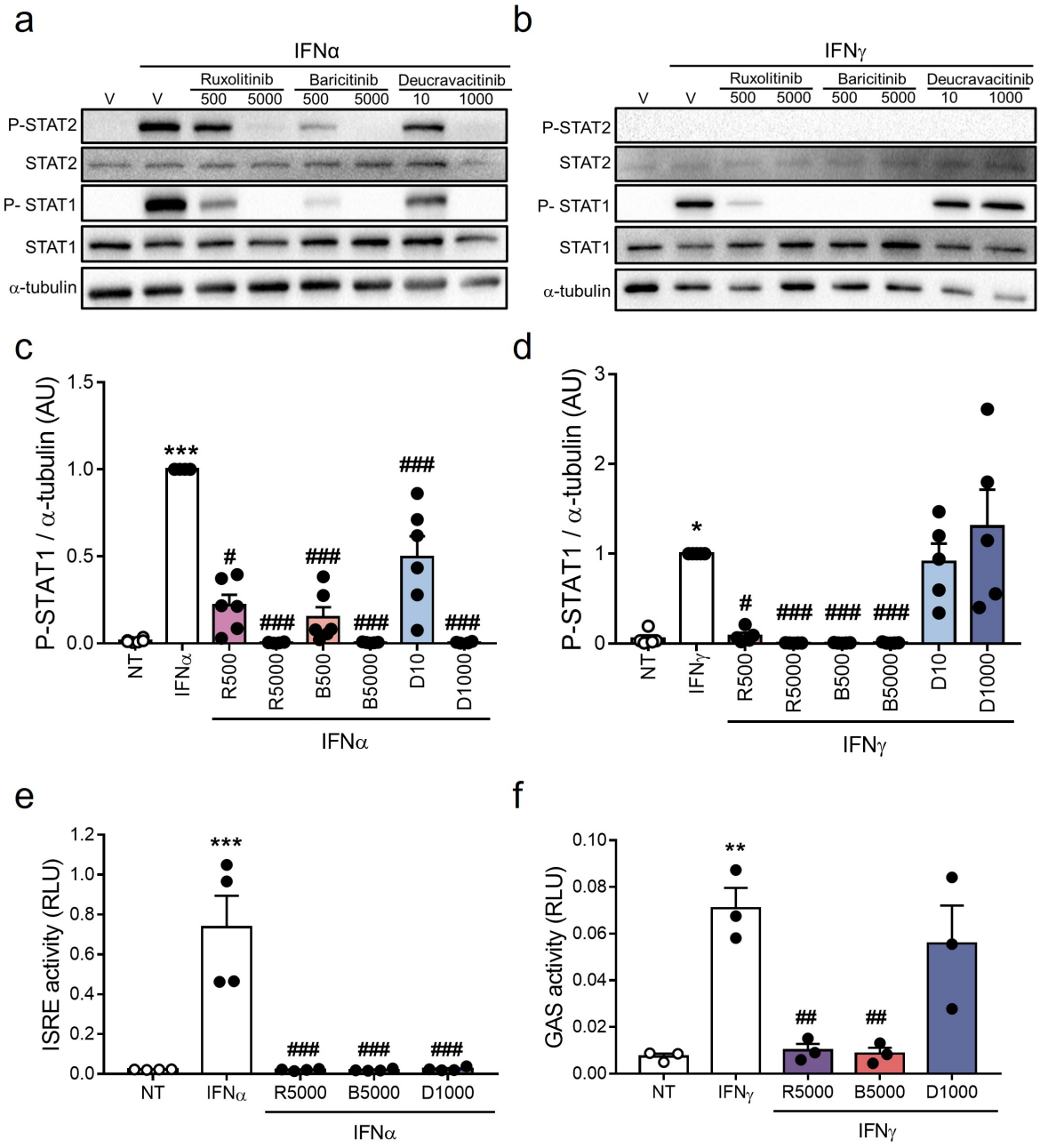
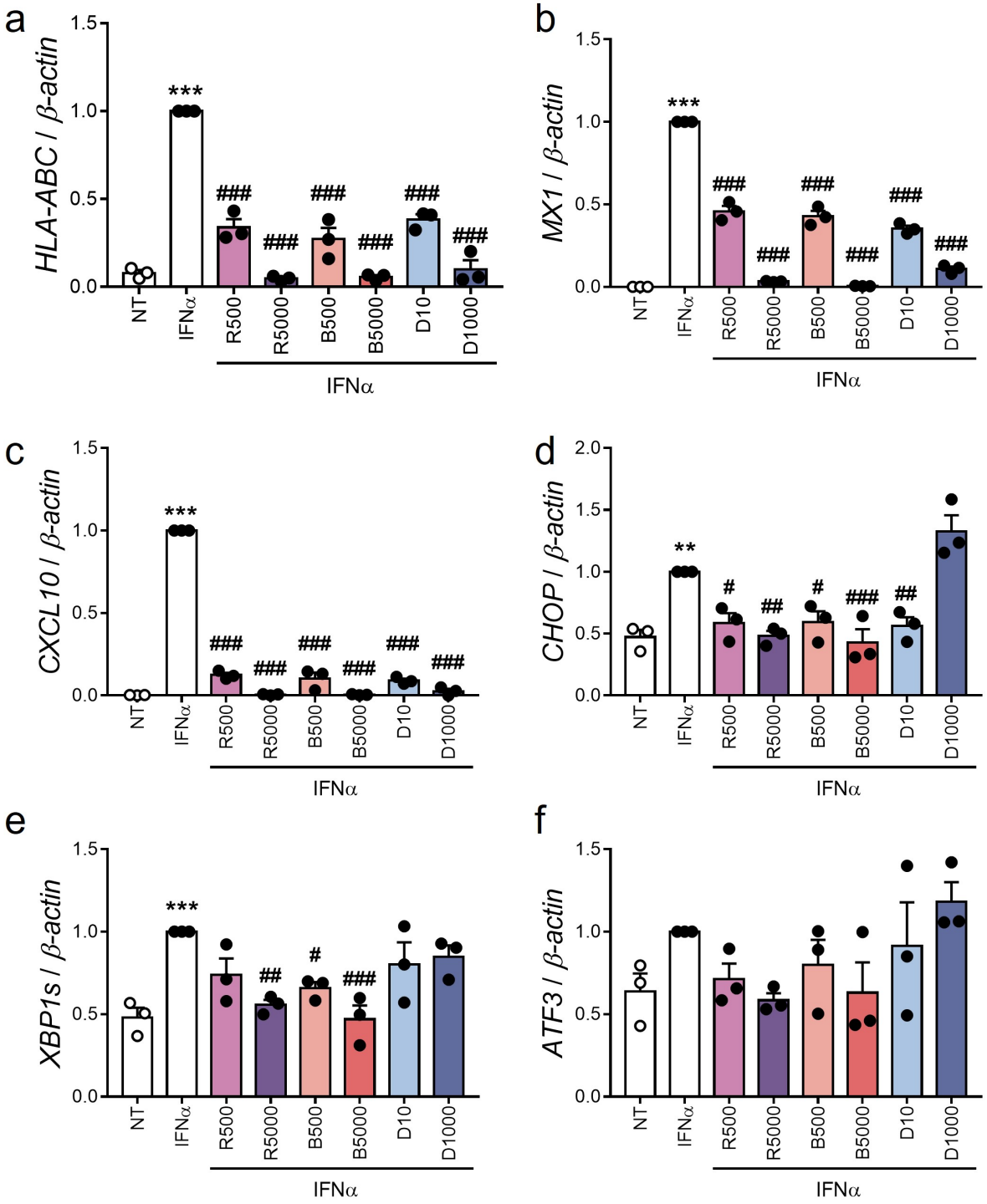


Figure 2. Deucravacitinib blocks IFNα- but not IFNγ-induced pathway. (**a-d**) EndoC-βH1 cells were treated with vehicle (V, white bars) or pre-treated with ruxolitinib (500 and 5000 nmol/l; R500 and R5000), baricitinib (500 and 5000 nmol/l; B500 and B5000), or deucravacitinib (10 and 1000 nmol/l; D10 and D1000) for 1 h. Afterwards, cells were left untreated (NT, white circles) or treated with either IFNα (1000 U/ml) (**a**, **c**) or IFNγ (1000 U/ml) (**b**, **d**) in the absence or presence of each inhibitor for 1 h. (**a-d**) Protein expression was measured by western blot. (**a**, **b**) Images are representative of five to six independent experiments. (**c**, **d**) Densitometry results are shown for P-

604 STAT1. Values were normalised to α-tubulin, and then to the value of IFNα (**c**) or IFNγ alone (**d**)
605 of each experiment (considered as 1). (**e**, **f**) EndoC-βH1 cells were transfected with a pRL-CMV
606 plasmid (used as internal control) plus either ISRE (**e**) or GAS (**f**) promoter reporter constructs.
607 After 48 h of recovery, cells were treated with vehicle (white bars) or pre-treated with ruxolitinib
608 (5000 nmol/l; R5000), baricitinib (5000 nmol/l; B5000), or deucravacitinib (1000 nmol/l; D1000)
609 for 1 h. Afterwards, cells were left untreated (NT, white circles) or treated with either IFNα (1000
610 U/ml) for 2 h (**e**) or IFNγ (1000 U/ml) for 24 h (**f**) in the absence or presence of each inhibitor.
611 Relative luciferase units (RLU) were measured by a luminescent assay. Data are means ± SEM of
612 three to six independent experiments. $^{*}p \leq 0.05$, $^{**}p \leq 0.01$ and $^{***}p \leq 0.001$ vs the respective
613 untreated (NT) (one-way ANOVA plus Dunnett's test). $^{\#}p \leq 0.05$, $^{\#\#}p \leq 0.01$ and $^{\#\#\#}p \leq 0.001$ vs IFNα
614 (**c**, **e**) or IFNγ (**d**, **f**) (one-way ANOVA plus Dunnett's test).



615 **Figure 3. Deucravacitinib abrogates IFNα-induced expression of ISGs but not ER stress**
616 **markers.** (**a-f**) EndoC-βH1 cells were treated with vehicle (white bars) or pre-treated with
617 ruxolitinib (500 and 5000 nmol/l; R500 and R5000), baricitinib (500 and 5000 nmol/l; B500 and
618 B5000), or deucravacitinib (10 and 1000 nmol/l; D10 and D1000) for 1 h. Afterwards, cells were

619 left untreated (NT) or treated with IFNα (1000 U/ml) in the absence or presence of each inhibitor
620 for 24 h. mRNA expression of *HLA-ABC* (**a**), *MX1* (**b**), *CXCL10* (**c**), *CHOP* (**d**), *XBP1s* (**e**), and
621 *ATF3* (**f**) was analysed by real-time PCR, normalised to β-actin and then to the value of IFNα alone
622 of each experiment (considered as 1). Data are means ± SEM of three independent experiments.
623 $^{**}p \leq 0.01$ and $^{***}p \leq 0.001$ vs the respective untreated (NT) (one-way ANOVA plus Dunnett's
624 test). $^{\#}p \leq 0.05$, $^{\#\#}p \leq 0.01$ and $^{\#\#\#}p \leq 0.001$ vs IFNα (one-way ANOVA plus Dunnett's test).

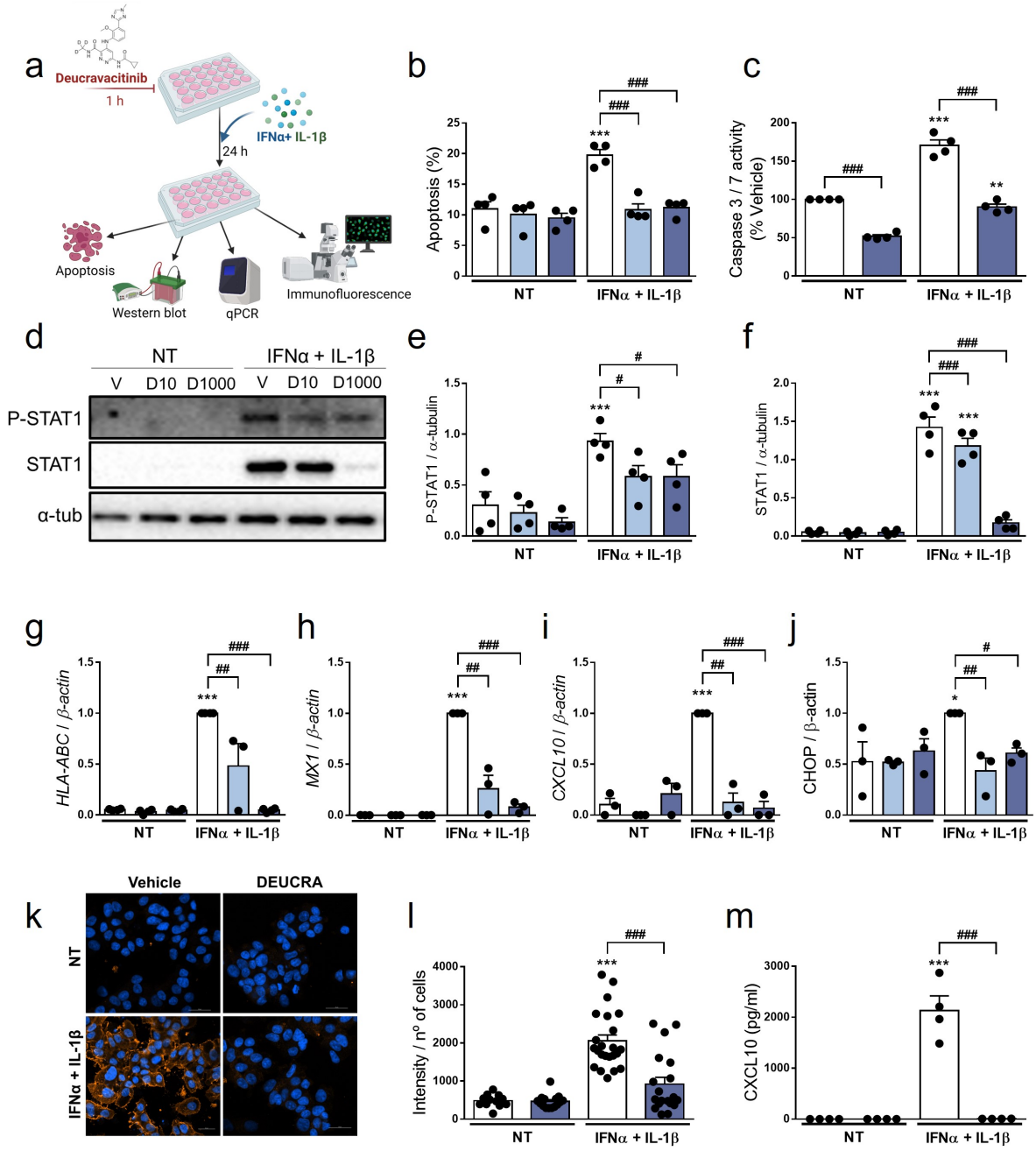


Figure 4. Pre-treatment with deucravacitinib prevents IFNα + IL-1β effects. (**a**) Experimental design of the pre-treatment with deucravacitinib and subsequent exposure to IFNα + IL-1β for 24 h. EndoC-βH1 cells were treated with vehicle (V, white bars) or pre-treated with deucravacitinib (10 [D10, soft blue circles] and 1000 nmol/l [D1000, dark blue circles]) for 1 h. Afterwards, cells were left untreated (NT) or treated with IFNα + IL-1β (1000 U/ml + 50 U/ml, respectively) in the absence or presence of deucravacitinib for 24 h. (**b**) Apoptosis was evaluated using Hoechst

631 33342/propidium iodide staining. (**c**) Caspase 3/7 activity was measured by a luminescent assay.
632 Results are expressed as % vehicle-treated cells in the absence of cytokines (NT). (**d-f**) Protein
633 expression was measured by western blot. (**d**) Images are representative of four independent
634 experiments. Densitometry results are shown for P-STAT1 (**e**) and P-STAT2 (**f**). Values were
635 normalised to α-tubulin. (**g-j**) mRNA expression of *HLA-ABC* (**g**), *CXCL10* (**h**), *MX1* (**i**), and
636 *CHOP* (**j**) was analysed by real-time PCR, normalised to β-actin and then to the value of Vehicle
637 treated with IFNα + IL-1β (considered as 1). (**k**, **l**) Immunocytochemistry analysis of MHC class
638 I (red) and Hoechst 33342 (blue) upon exposure to IFNα + IL-1β in the absence (white bars) or
639 presence of deucravacitinib (dark blue bars) for 24 h. Representative images of three independent
640 experiments (12–23 images/coverslip) (**g**) and quantification (**h**) are shown. (**j**) CXCL10 secreted
641 to the medium was determined by ELISA. Data are means ± SEM of three to four independent
642 experiments. $^{**}p \leq 0.01$ and $^{***}p \leq 0.001$ vs the respective untreated (NT) (two-way ANOVA plus
643 Sidak's test). $^{\#}p \leq 0.05$, $^{\#\#}p \leq 0.01$ and $^{\#\#\#}p \leq 0.001$, as indicated by bars (two-way ANOVA plus
644 Dunnett's test).

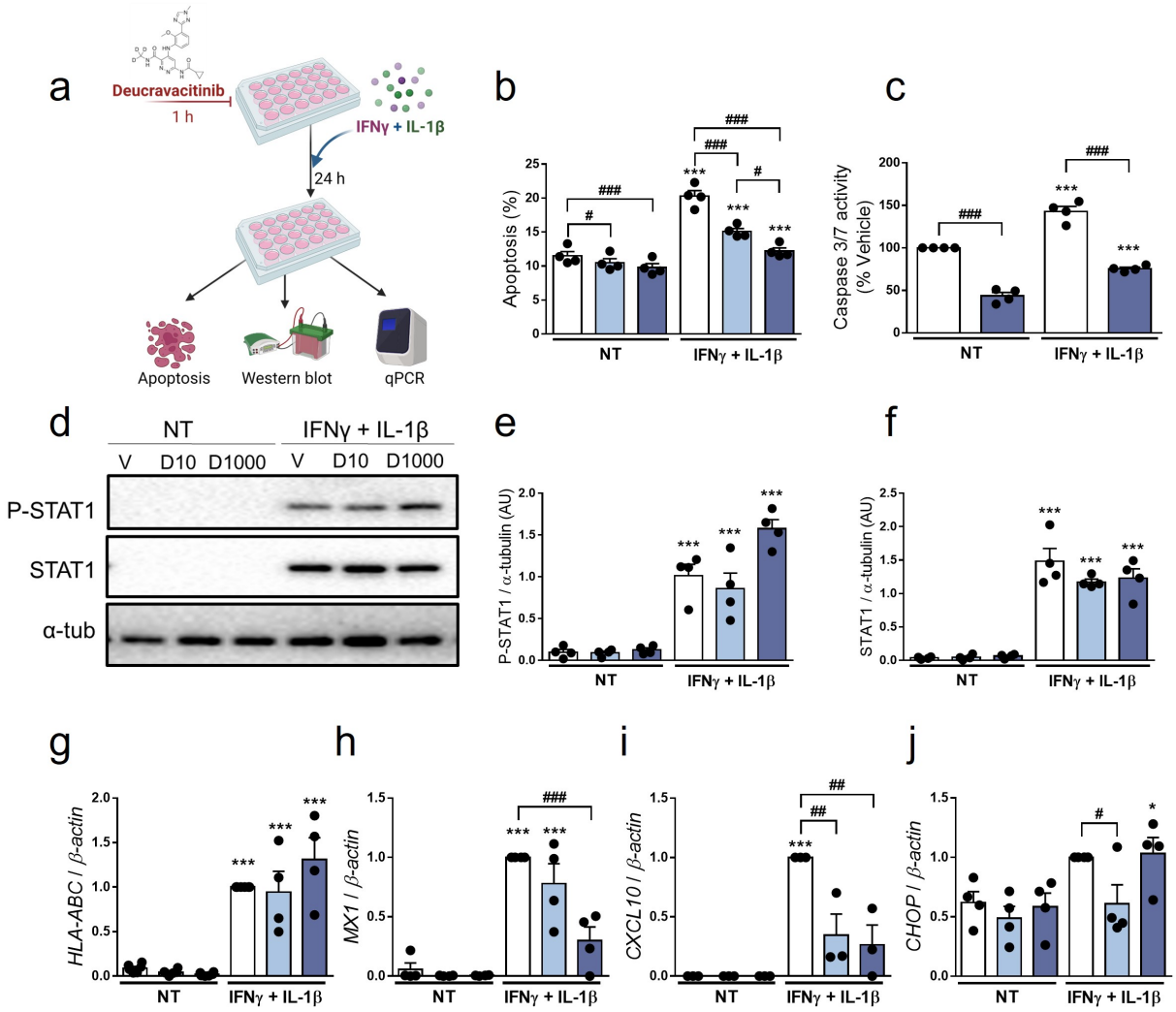


Figure 5. Pre-treatment with deucravacitinib prevents IFNγ + IL-1β effects. (**a**) Experimental design of the pre-treatment with deucravacitinib and subsequent exposure to IFNγ + IL-1β for 24 h. EndoC-βH1 cells were treated with vehicle (V, white bars) or pre-treated with deucravacitinib (10 [D10, soft blue circles] and 1000 nmol/l [D1000, dark blue circles]) for 1 h. Afterwards, cells were left untreated (NT) or treated with IFNγ + IL-1β (1000 U/ml + 50 U/ml, respectively) in the absence or presence of deucravacitinib for 24 h. (**b**) Apoptosis was evaluated using Hoechst 33342/propidium iodide staining. (**c**) Caspase 3/7 activity was measured by a luminescent assay. Results are expressed as % vehicle-treated cells in the absence of cytokines (NT). (**d-f**) Protein expression was measured by western blot. Images are representative of four independent experiments (**d**). Densitometry results are shown for P-STAT1 (**e**) and P-STAT2 (**f**). Values were

655 normalised to α-tubulin. (**g-j**) mRNA expression of *HLA-ABC* (**g**), *CXCL10* (**h**), *MX1* (**i**), and
656 *CHOP* (**j**)was analysed by real-time PCR, normalised to β-actin and then to the value of Vehicle
657 treated with IFNγ + IL-1β (considered as 1). Data are means ± SEM of three to four independent
658 experiments. *$p \leq 0.05$, **$p \leq 0.01$ and ***$p \leq 0.001$ vs the respective untreated (NT) (two-way
659 ANOVA plus Sidak's test). $^{\#}p \leq 0.05$, $^{\#\#}p \leq 0.01$ and $^{\#\#\#}p \leq 0.001$, as indicated by bars (two-way
660 ANOVA plus Dunnett's test).

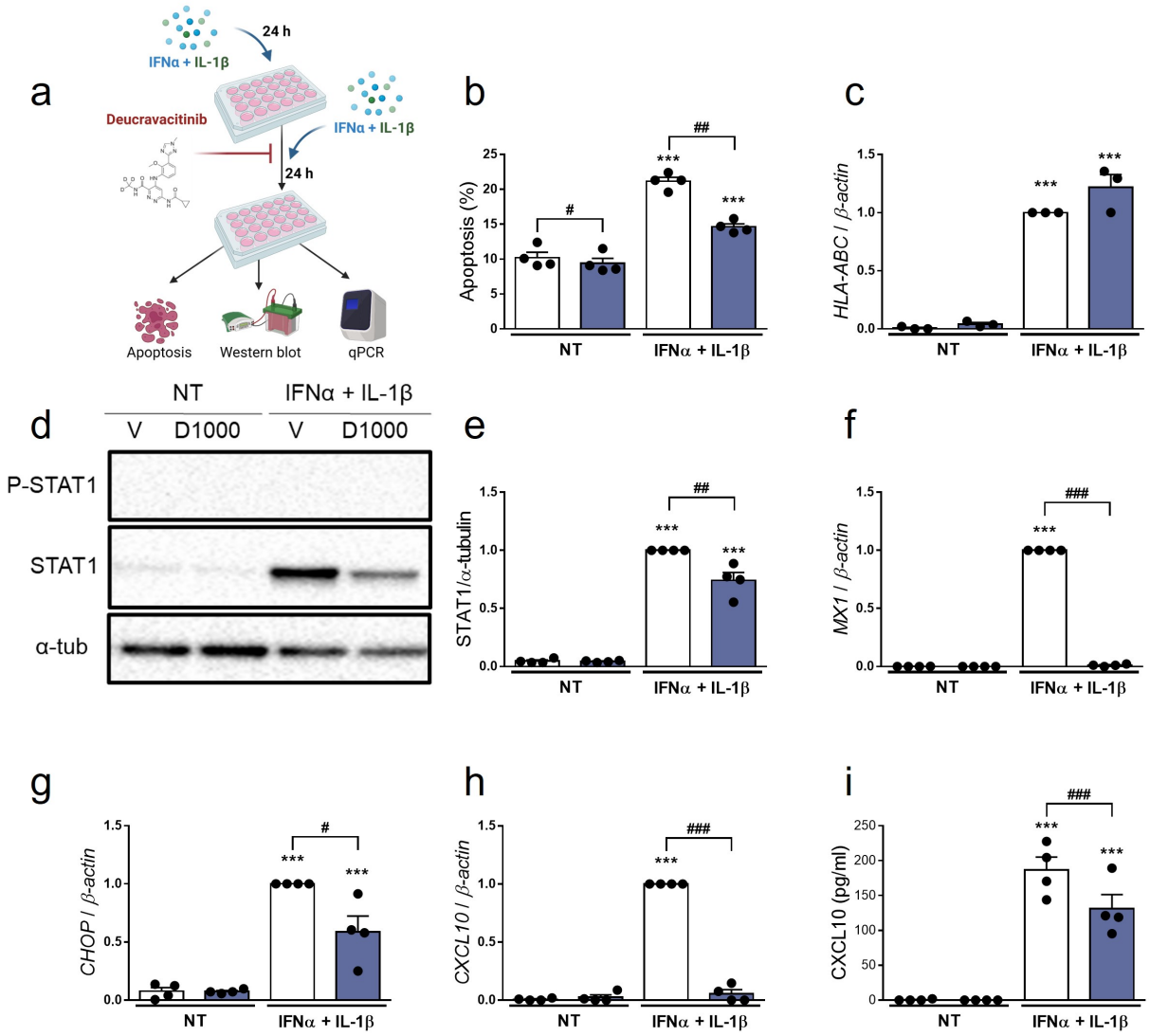


Figure 6. Treatment with deucravacitinib partially reverts IFNα + IL-1β-induced changes. (**a**) Experimental design of the pre-treatment with IFNα + IL-1β and subsequent exposure to IFNα + IL-1β in the presence of deucravacitinib for 24 h. EndoC-βH1 cells were left untreated (NT) or pre-treated with IFNα + IL-1β (1000 U/ml + 50 U/ml, respectively) for 24 h. Afterwards, cells were treated with vehicle (V, white bars) or 1000 nmol/l deucravacitinib (D1000, dark blue bars) in the absence (NT) or presence of IFNα + IL-1β for 24 h. (**b**) Apoptosis was evaluated using Hoechst 33342/propidium iodide staining. (**c**, **f-h**) mRNA expression of *HLA-ABC* (**c**), *MX1* (**f**), *CHOP* (**g**), and *CXCL10* (**h**) was analysed by real-time PCR, normalised to β-actin and then to the value of Vehicle treated with IFNα + IL-1β (considered as 1). (**d**, **e**) Protein expression was measured by western blot. (**d**) Images are representative of four independent experiments. (**e**)

671 Densitometry results are shown for STAT1. Values were normalised to α-tubulin. (**i**) CXCL10
672 secreted to the medium was determined by ELISA. Data are means ± SEM of three to four
673 independent experiments. $^{**}p \leq 0.01$ and $^{***}p \leq 0.001$ vs the respective untreated (NT) (two-way
674 ANOVA plus Sidak's test). $^{\#}p \leq 0.05$, $^{\#\#}p \leq 0.01$ and $^{\#\#\#}p \leq 0.001$, as indicated by bars (two-way
675 ANOVA plus Dunnett's test).

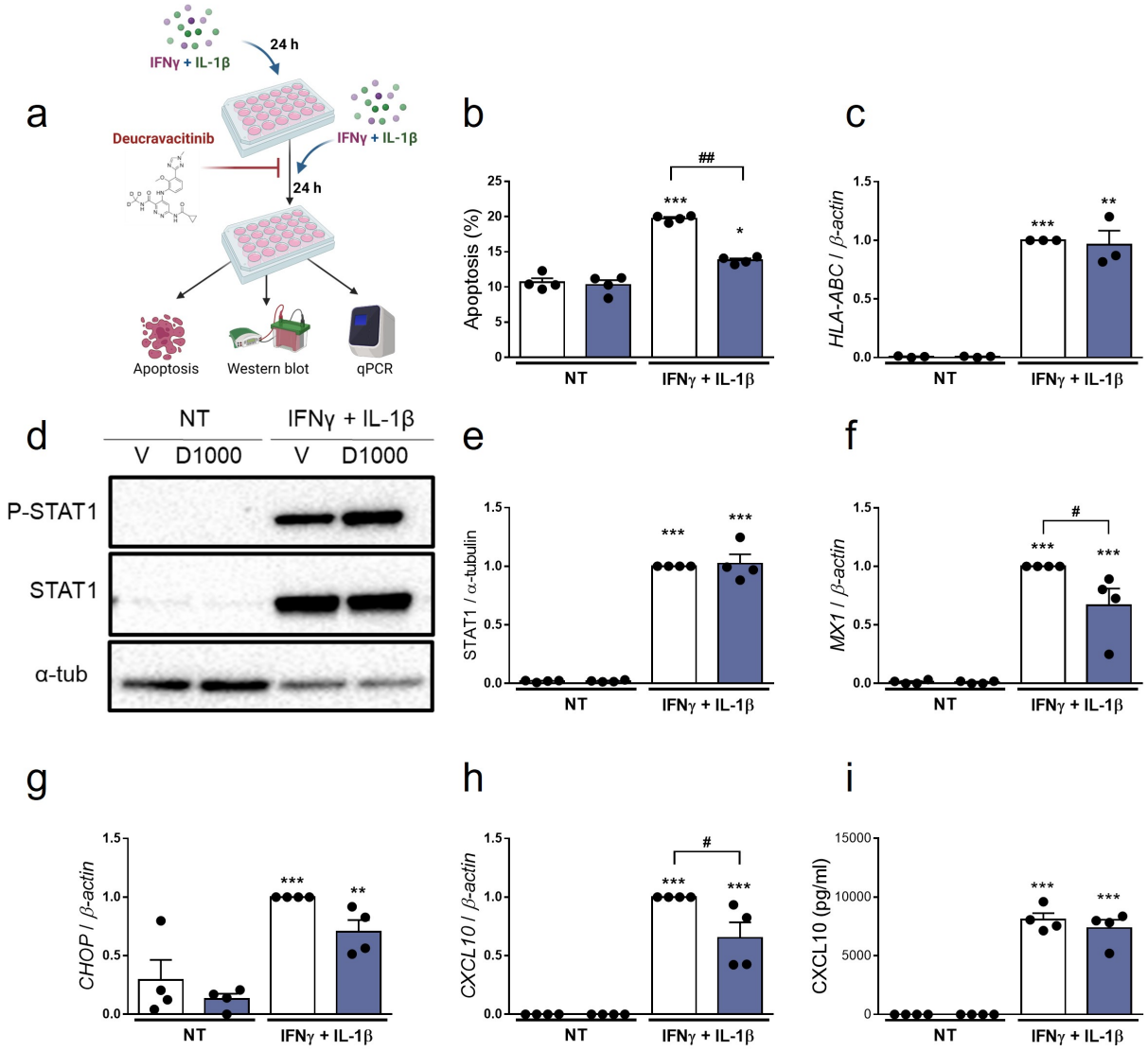


Figure 7. Treatment with deucravacitinib partially reverts IFNγ + IL-1β-induced changes. (**a**) Experimental design of the pre-treatment with IFNγ + IL-1β and subsequent exposure to IFNγ + IL-1β in the presence of deucravacitinib for 24 h. EndoC-βH1 cells were left untreated (NT) or pre-treated with IFNγ + IL-1β (1000 U/ml + 50 U/ml, respectively) for 24 h. Afterwards, cells were treated with vehicle (V, white bars) or 1000 nmol/l deucravacitinib (D1000, dark blue bars) in the absence (NT) or presence of IFNγ + IL-1β for 24 h. (**b**) Apoptosis was evaluated using Hoechst 33342/propidium iodide staining. (**c**, **f-h**) mRNA expression of *HLA-ABC* (**c**), *MX1* (**f**), *CHOP* (**g**), and *CXCL10* (**h**) was analysed by real-time PCR, normalised to β-actin and then to the value of Vehicle treated with IFNγ + IL-1β (considered as 1). (**d**, **e**) Protein expression was measured by western blot. (**d**) Images are representative of four independent experiments. (**e**)

686 Densitometry results are shown for STAT1. Values were normalised to α-tubulin. (**i**) CXCL10
687 secreted to the medium was determined by ELISA. Data are means ± SEM of three to four
688 independent experiments. *$p \leq 0.05$, **$p \leq 0.01$ and ***$p \leq 0.001$ vs the respective untreated (NT)
689 (two-way ANOVA plus Sidak's test). $^{\#}p \leq 0.05$ and $^{\#\#}p \leq 0.01$, as indicated by bars (two-way
690 ANOVA plus Dunnett's test).