Molecular and Cellular Biochemistry
https://doi.org/10.1007/s11010-025-05435-4

IGF-1 protects against acute pancreatitis by suppressing NF-κB activation through the β-arrestin1/STAT3 pathway

Xiaoli Huang[1,2,3] · Li Tao[1,2,3] · Huiling Liu[1,2,3] · Hongju Luo[1,2,3] · Yunwei Guo[1,2,3]

Received: 5 April 2025 / Accepted: 28 October 2025

Abstract

Acute pancreatitis (AP) is a prevalent inflammatory condition with an upward trend in incidence in recent years. Recent studies have demonstrated the important role of IGF-1 and β-arrestin-1 in inflammation, but their role in AP remains unexplored. Our study explored the role of IGF-1 in acute pancreatitis (AP) using caerulein-induced AP models in wild-type (WT) and *β-arrestin1*-knockout (KO) mice treated with IGF-1, picropodophyllotoxin (PPP, an IGF-1R inhibitor), Bay11708 (an NF-κB inhibitor), and the STAT3 inhibitor VI. Caerulein injection induced AP, characterized by elevated serum amylase levels, increased MPO activity, and pancreatic pathology. IGF-1 treatment reduced the severity of AP, whereas PPP worsened it. β-arrestin1 deficiency exacerbated pancreatitis and abolished the protective effect of IGF-1. NF-κB and STAT3 were involved in the protective mechanism of IGF-1 through β-arrestin1 regulation. Inhibiting NF-κB or STAT3 altered the protective effect of IGF-1 in AP. In vitro, the protective effect of IGF-1 against caerulein-induced injury was demonstrated in AR42J cells. This study revealed that IGF-1 protected against AP by suppressing NF-κB activation through the β-arrestin1/STAT3 pathway, providing new insights into the role of IGF-1 in AP and potential therapeutic targets.

Graphical abstract

Schematic diagram depicting the role of IGF-1 in acute pancreatitis. IGF-1 protected against acute pancreatitis by suppressing NF-κB activation through the β-arrestin1/STAT3 signaling pathway

Xiaoli Huang and Li Tao contributed equally to this work.

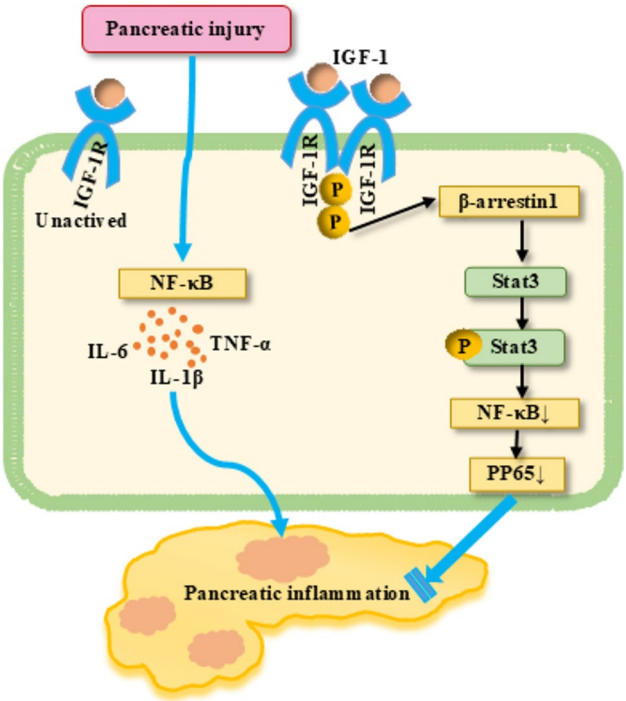
✉ Yunwei Guo
guoyw@mail.sysu.edu.cn

[1] Department of Gastroenterology, The Third Affiliated Hospital of Sun Yat-Sen University, 600 Tianhe Road, Guangzhou 510630, Guangdong, P. R. China

[2] Alcoholic Liver Disease Center, The Third Affiliated Hospital of Sun Yat-Sen University, Guangzhou, Guangdong, P. R. China

[3] Guangdong Provincial Key Laboratory of Liver Disease Research, Guangzhou, Guangdong, P. R. China

Published online: 11 November 2025



Keywords Pancreatitis · IGF-1 · NF-κB · β-arrestin1 · STAT3

Abbreviations

GPCR	G-protein-coupled receptor
MAPKs	Mitogen-activated protein kinases
STAT	Signal transducer and activator of transcription
IGF-1	Insulin-like growth factor-1
IGF-1R	Insulin-like growth factor-1 receptor
NF-κB	Nuclear factor-κB

Introduction

Acute pancreatitis (AP) is a prevalent inflammatory condition whose incidence has exhibited an upward trend in recent years. Mild acute pancreatitis may be self-limiting and may not necessitate any medical intervention. Conversely, severe acute pancreatitis represents a life-threatening condition with a high mortality rate, with 30 to 50% of patients succumbing to the disease [1]. Several studies have indicated that damaged acinar cells may contribute to premature intrapancreatic zymogen activation, which is thought to be a key factor in the onset of this debilitating disease [2–5]. Additionally, for more than a decade, NF-κB activation has been observed to occur early in the onset of pancreatitis, in parallel with trypsinogen activation [6, 7]. Furthermore, studies have indicated that damaged acinar cells may produce inflammatory mediators that recruit and activate leukocytes, which in turn amplify and expand the disease area [8]. This could lead to further acinar cell injury and a significant increase in the levels of various proinflammatory cytokines, such as TNF-α, IL-1β, IL-6, and CXCL1, which could in turn activate inflammatory cells [9, 10]. Despite extensive research, the precise pathological mechanisms underlying the development of acute pancreatitis remain unclear. It is therefore necessary to investigate the specific pathogenesis of this complex disease process.

Insulin-like growth factor-1 (IGF-1) plays a pivotal role in tissue remodeling and development and profoundly affects cell growth, differentiation, proliferation and apoptosis [11–13]. The majority of the biological activities of IGF-1 are mediated by the IGF-1 receptor (IGF-1R) and its downstream effectors, which include the well-characterized mitogen-activated protein kinase (MAPK) signaling networks [14, 15]. Furthermore, IGF-1/IGF-1R has been demonstrated to regulate numerous additional signaling mediators, including NF-κB, PI3K/AKT and STATs, to modulate cellular responses to external stimuli [5, 16, 17]. Previous studies have demonstrated that exogenous IGF-1 can mitigate pancreatic injury and facilitate pancreatic recovery in acute experimental pancreatitis [18]. Nevertheless, the precise mechanism of action of IGF-1 in AP remains unclear [19].

IGF-1Rs are members of the receptor tyrosine kinase (RTK) family. IGF-1 binds to this receptor, resulting in autophosphorylation, which enables the recruitment of adaptor

proteins capable of activating signaling cascades, including the JAK/STAT pathway, in a multitude of cell types [16]. The function of β-arrestins in IGF-1R ubiquitination has only recently been elucidated, as their activities have typically been linked to G protein-coupled receptors (GPCRs), also known as seven transmembrane receptors (7TMRs) [20–22]. Among the four members of the β-arrestin family, β-arrestin1 (formerly known as arrestin-2) and β-arrestin2 (formerly known as arrestin-3) are cytosolic proteins that are expressed ubiquitously and are recruited to and mediate the desensitization of GPCRs upon agonist binding [23–25]. Although β-arrestins were initially described as negative regulators of GPCR effects, more recent studies have demonstrated that the recruitment of β-arrestin to agonist-occupied receptors may also result in the activation of a range of signaling pathways, including the NF-κB, MAPK, PI3K/AKT and STAT pathways [20, 26]. β-arrestin1 is recruited to the insulin-like growth factor 1 receptor (IGF-1R) upon binding to IGF-1, where it regulates the downstream signaling networks of the receptor, thereby influencing cellular function [27]. Given their pivotal function in numerous cellular processes, β-arrestins play a vital role in the pathogenesis of a wide range of diseases, including Parkinson's disease, multiple sclerosis, cardiovascular disease, rheumatoid arthritis, sepsis, and colitis [28–30]. Our previous study demonstrated that β-arrestin1 is also involved in caerulein-induced acute pancreatitis [31]. In light of these findings, we hypothesized that β-arrestin1 may also participate in the IGF-1 receptor-mediated signaling pathway during the protective effects of IGF-1 in acute pancreatitis.

Materials and methods

Mice

The procedures for all animal experiments were approved by the Institutional Animal Care and Use Committee at The Third Affiliated Hospital of Sun Yat-Sen University. *β-arrestin1* wild-type (WT) and *β-arrestin1* knockout (*β-arrestin1*-KO) littermates on the C57BL/6 background were generated from heterozygous intercrosses (kindly provided by Dr. R. J. Lefkowitz, Duke University Medical Center, Durham, NC, USA). Eight- to ten-week-old male mice (20–25 g) were used for all experiments. The mice were housed in microisolator cages under specific pathogen-free conditions, with lights on from 7:00 am to 7:00 pm (12:12-h light/dark cycle), and allowed access to water and food ad libitum.

Induction of acute pancreatitis

The mice were injected i.p. with caerulein (50 μg/kg in 0.2 ml of PBS; Sigma, St Louis, MO, USA) repeatedly every 1 h for a total of 10 injections. In some experiments, mice were injected with 80 μg/kg IGF-1 (Sigma), 100 mg/kg picropodophyllotoxin (PPP; an IGF-1R inhibitor; Sigma), 200 μg/kg Bay11708 (BAY; an NF-κB inhibitor; Calbiochem, La Jolla, CA) or 5 mg/kg STAT3 inhibitor VI, S3I-201 (STAT3 inhibitor; S3I-201; Sigma), which was administered 30 min prior to caerulein treatment. The mice were observed for up to 4, 8, 12 and 24 h and were anesthetized and subsequently euthanized. The entire pancreas was meticulously removed and then stored at -80 °C for subsequent protein or RNA analysis. For the preparation of paraffin sections, the pancreas from each group was carefully isolated and immediately fixed in 10% neutral buffered formalin before being embedded.

Histopathological analysis

Pancreatic tissue Sects. (4 μm) were subjected to histological analysis, with staining conducted using the hematoxylin–eosin (HE) method. The histological scores were determined in a blinded manner on the basis of previously described criteria. The entire pancreas of mice from each group was examined and semiquantified on the basis of necrosis, vacuolization, inflammation, and edema. The entire section, representing a minimum of 100 fields, was examined for each sample and scored on a scale of 0–3 (0 being normal and 3 being severe). Finally, each group was summarized for the total score.

Measurement of serum amylase levels

Following anesthesia, arterial blood samples were obtained from the mice to determine serum amylase levels. The concentration of serum amylase was determined using an enzyme-linked immunosorbent assay (ELISA) kit (BioAssay Systems, Hayward, CA) in accordance with the manufacturer's instructions.

MPO activity estimation

Neutrophil sequestration in the pancreas was quantified by measuring tissue MPO activity. For these measurements, tissue samples were thawed, homogenized in 50 mM phosphate buffer (pH 7.4) containing protease inhibitors and centrifuged (16,000 × g for 10 min at 4 °C). The resulting pellet was resuspended in 50 mM phosphate buffer (pH 6.0) containing 0.5% hexadecyltrimethylammonium bromide and 10 mM EDTA. The suspension was subjected to three

cycles of freezing and thawing and was further disrupted by sonication for 40 s. The sample was then centrifuged (16,000 × g for 15 min, 4 °C), after which the supernatant was used for the MPO assay. The reaction mixture consisted of the supernatant, 2 mM tetramethylbenzidine, 60 mM sodium phosphate buffer (pH 5.4), and 0.3 mM hydrogen peroxide. This mixture was incubated at 37 °C for 2 min, the reaction was terminated with 2 M H2SO4, the absorbance was measured at 450 nm, and MPO activity was expressed as units per milligram of protein.

Immunohistochemical staining

For immunohistochemical (IHC) staining, paraffin-embedded pancreatic sections were deparaffinized, rehydrated, treated with 3% hydrogen peroxide, fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) for 15 min, permeabilized with 1% Triton X-100 for 20 min and then washed three times with PBS. The target proteins IGF-1 (Santa Cruz Biotechnology, Heidelberg, Germany), p-IGF-1R (Cell Signaling Technology, Danvers, MA, USA), Ly6G and β-arrestin1 (Abcam, Cambridge, MA, USA) were then detected using secondary antibodies, followed by the ABC staining system, and the sections were counterstained with hematoxylin.

Real-time polymerase chain reaction (Real-time PCR)

Total RNA was isolated from mice pancreas samples using the RNAgents Total RNA Isolation System (Promega, Madison, WI, USA) according to the manufacturer's instructions. First-strand cDNA was synthesized using Superscript reverse transcriptase (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Real-time polymerase chain reaction (PCR) was performed on a Chromo 4 Detector System (MJ Research, Sierra Point, CA, USA) using gene-specific primers and DyNAmo SYBR Green Master Mix (Finnzymes, Finland). Relative RNA was amplified using the following primers: IGF-1 exon 3F, 5'-TCA TGT CGT CTT CAC ACC TCT TCT-3' and IGF-1 exon 4R, 5'-CCA CAC ACG AAC TGA AGA GCA T-3'; TNF-α exon 3F, 5'-CTT CCT CTC ATT CCT GCT TGT G-3' and TNF-α exon 4R, 5'-CTG GAA GAC TCC TCC CAG GTA TAT-3'; IL-1β exon 3F, 5'-TTG ACG GAC CCC AAAAGA T-3' and IL-1β exon 4R, 5'-GAA GCT GGA TGC TCT CAT CTG-3'; IL-6 exon 3F, 5'-ATC CAG TTG CCT TCT TGG GAC TGA-3' and IL-6 exon 4R, 5'-TAA GCC TCC GAC TTG TGA AGT GGT-3'; IL-10 exon 3F, 5'-TGC TAT GCT GCC TGC TCT TA-3' and IL-10 exon 4R, 5'-TCA TTT CCG ATA AGG CTT GG-3'. As an internal control, β-actin expression was also quantified in each sample using the sense primer 5'-GTG GGC CGC TCT AGG CAC CA-3' and the antisense primer 5'-CGG TTG GCC TTA GGG TTC AGG GGG G-3'. Six mouse tissues were used in each group.

Western blotting

Total protein extraction was analyzed by Western blotting. The antibodies used for Western blotting included antibodies recognizing β-actin (Santa Cruz, CA, USA), IGF-1 (Proteintech), p-IGF-1R and IGF-1R (Cell Signaling Technology), p65 (Santa Cruz), p-p65 (Cell Signaling Technology), β-arrestin1, STAT3 and p-STAT3 (all from Abcam). Appropriate horseradish peroxidase-conjugated secondary antibodies were used to detect the primary antibody-antigen complexes. The signal was detected using enhanced chemiluminescence (ECL) Western blotting detection reagents (Amersham Pharmacia Biotech, Piscataway, NJ, USA). The signals were quantified by densitometry, and the results are expressed as a ratio to the densitometric units of the loading control.

Cell culture, drug administration and small interfering RNA (siRNA) treatment

The pancreatic acinar cell line (AR42J) was obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). Cells were cultured in EMEM supplemented with 10% heat-inactivated fetal bovine serum, 100 units/ml penicillin and 100 μg/ml streptomycin in a humidified incubator at 37 °C with 5% CO2. Caerulein (along a concentration gradient such as 0, 10, 20, 40, 80, 100 μmol/L or at time points such as 0, 2, 4, 8, 12, 24 h; Sigma) was added after these cells had grown to a density of 90%. The other drugs—IGF-1 (100 ng/ml; Sigma), Bay11708 (BAY; 20 μmol/L; Calbiochem) or the STAT3 inhibitor VI, S3I-201 (S3I-201; 100 μmol/L; Sigma)—were added 30 min before caerulein treatment (80 μmol/L for 8 h). For siRNA treatment, cells were transfected with 20 μM β-arrestin1 with an RNA oligo kit (GenePharma, China) according to the manufacturer's instructions, and after incubation for 24 h, the transfection medium was replaced with regular culture medium before caerulein administration.

Cell viability assay

To evaluate cell viability after exposure to varying concentrations of S3I-201, a cell counting kit-8 (CCK-8) assay (DOJINDO, CK04) was utilized. AR42J cells were plated in 96-well plates at a density of 1×10^4 cells per well. Following an initial adhesion and recovery period, the cells were treated with S3I-201 at concentrations of 0, 25, 50,

100, 150, and 200 μmol/L for 8 h. The culture medium was subsequently replaced with 100 μL of fresh medium supplemented with 10 μL of CCK-8 reagent. The cells were incubated for an additional hour at 37 °C in a 5% CO_2 atmosphere. Cell viability was determined by measuring the absorbance at 450 nm using a microplate reader (Thermo Scientific, Varioskan Flash). The results indicated that treatment with 100 μmol/L S3I-201 did not significantly affect cell viability.

Statistical analysis

Statistical comparisons were performed using SPSS 27.0. All data are expressed as the mean ± SD. Comparisons of multiple groups were analyzed by one-way analysis of variance (ANOVA) followed by Bonferroni post hoc correction. Differences between two groups were determined by Student's t test. $P<0.05$ was considered to indicate statistical significance.

Results

The expression of p-IGF-1R in the pancreas following a caerulein challenge

To investigate the role of IGF-1 in the process of acute pancreatitis, we established an in vivo mouse model of acute pancreatitis via intraperitoneal injection of caerulein. Real-time PCR analysis of pancreatic tissue samples revealed that the level of IGF-1 mRNA increased at the designated time points following the caerulein challenge (Fig. 1A). However, no significant upregulation of IGF-1 expression was detected by either Western blotting (WB) or immunohistochemical (IHC) staining (Fig. 1B and C). IGF-1 regulates cellular function through binding to its high-affinity receptor, IGF-1R, which can be phosphorylated to activate a number of downstream signal transduction pathways. Western blotting analysis was performed to further substantiate these findings, revealing significant upregulation of p-IGF-1R expression in the pancreas over time following caerulein injection (Fig. 1D). In contrast, total IGF-1R expression did not appear to be affected. Our previous research demonstrated that β-arrestin1 has a protective effect on acute pancreatitis, and its expression was markedly suppressed following caerulein injection. Additionally, β-arrestin1 protein expression decreased gradually at the specified time points (Fig. 1D), with undetectable levels after 36 h of caerulein stimulation (data not shown). P-IGF immunochemical staining with anti-p-IGF-IR antibodies revealed a similar trend to that observed by Western blotting, whereby the expression of p-IGF-IR markedly increased in pancreatic tissues over time following caerulein injection (Fig. 1C and F). Histopathological analysis revealed that following stimulation for 4 h, the interstitial tissues exhibited mild edema and vacuoles in acinar cells (Fig. 1C). With time, the tissues exhibited discernible morphological alterations, including moderate edema with a gradual increase in leukocyte infiltration and acinar cell necrosis. By 24 h post-challenge, the severity of necrosis, vacuolization and inflammatory cell infiltration had increased, and the preserved architecture of the acinar cells had undergone significant change (Fig. 1C). A histological score was assigned to each group of mice on the basis of the evaluation of necrosis, vacuolization, inflammation, and edema observed in HE-stained pancreas sections. The results demonstrated that the score increased with increasing stimulation duration (Fig. 1E). Moreover, the serum amylase and pancreatic MPO activity levels began to increase in accordance with the onset of histopathological alterations in pancreatic acinar cells (Fig. 1G, H), which are characteristic of acute pancreatitis. On the basis of these findings, we can conclude that p-IGF-1R expression was upregulated in the pancreases of mice with acute pancreatitis induced by caerulein.

The inhibition of IGF-1R aggravated caerulein-induced acute pancreatitis

Although previous studies have indicated that IGF-1 may have a protective effect against acute pancreatitis, their conclusions were inconclusive [18, 19, 32]. Our previous data indicated that p-IGF-1R expression is significantly upregulated in the pancreas of subjects with acute pancreatitis. The objective of this study was to determine the effects of administering an additional dose of IGF-1 and inhibiting the function of IGF-1R on the development of pancreatitis induced by caerulein. Histological examination revealed that the administration of the IGF-1R inhibitor picropodophyllotoxin at a dosage of 100 mg/kg 30 min prior to caerulein treatment resulted in a notable exacerbation of pancreatitis symptoms, characterized by increased edema and leukocyte infiltration. Conversely, the administration of 80 μg/kg IGF-1 led to a significant attenuation of caerulein-induced pancreatitis (Fig. 2A). The results of the histopathological analysis indicated that the histological score followed the same trend as that of the morphological changes (Fig. 2B). Moreover, serum amylase, a crucial marker of pancreatic acinar cell injury, was notably elevated following picropodophyllotoxin administration. Conversely, IGF-1 treatment resulted in a pronounced reduction in serum amylase levels (Fig. 2C). Furthermore, we demonstrated that the activity of the biochemical marker of neutrophil infiltration MPO was elevated following the inhibition of IGF-1R function during pancreatitis but was reduced following additional IGF-1

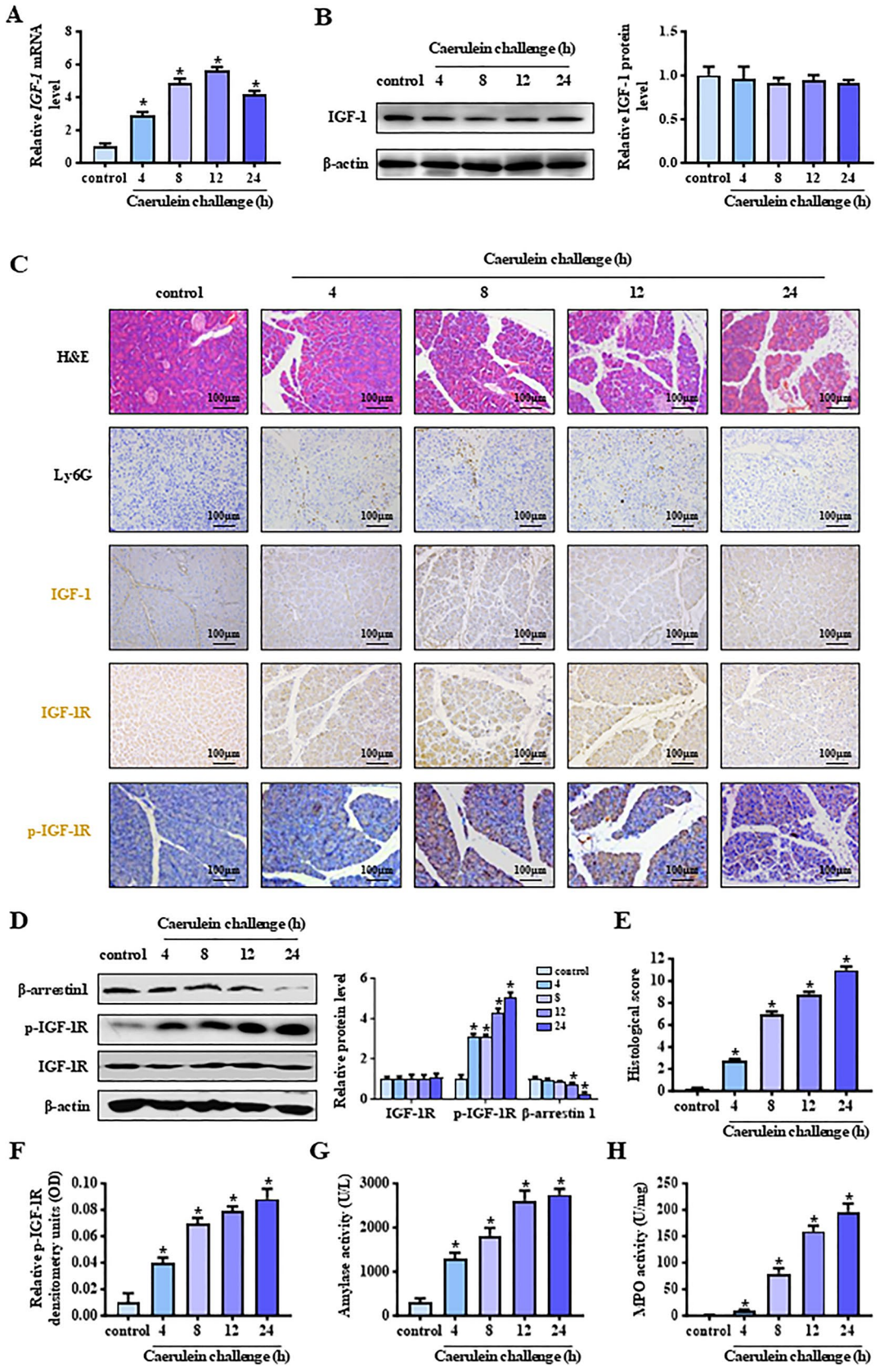

A
Relative IGF-1 mRNA level
control 4 8 12 24
Caerulein challenge (h)
B
Caerulein challenge (h)
control 4 8 12 24
IGF-1
β-actin
Relative IGF-1 protein level
control 4 8 12 24
Caerulein challenge (h)
C
Caerulein challenge (h)
control 4 8 12 24
H&E
Ly6G
IGF-1
IGF-1R
p-IGF-1R
100μm
D
Caerulein challenge (h)
control 4 8 12 24
β-arrestin1
p-IGF-1R
IGF-1R
β-actin
Relative protein level
control
4
8
12
24
IGF-1R
p-IGF-1R
β-arrestin 1
E
Histological score
control 4 8 12 24
Caerulein challenge (h)
F
Relative p-IGF-1R densitometry units (OD)
control 4 8 12 24
Caerulein challenge (h)
G
Amylase activity (U/L)
control 4 8 12 24
Caerulein challenge (h)
H
MPO activity (U/mg)
control 4 8 12 24
Caerulein challenge (h)

Fig. 1 The expression of p-IGF-1R in the pancreas following caerulein challenge. **A** The relative *IGF-1* mRNA levels in the indicated pancreas sections from WT mice after caerulein challenge were determined by real-time PCR. $n=6$ in each group; data are presented as the mean ± SD. $^*P<0.05$ versus the control group. **B** IGF-1 protein expression in the pancreas was determined by Western blotting at the indicated time points after caerulein challenge. β-actin was used as the loading control. **C** HE staining and immunohistochemical staining for Ly6G, IGF-I, IGF-IR, and p-IGF-IR in the indicated pancreas sections from WT mice after caerulein challenge at the indicated time points are shown (brown, × 200). **D**β-arrestin1, p-IGF-IR and IGF-1R protein expression levels in the pancreas were determined by Western blotting at the indicated time points after caerulein challenge. β-actin was used as the loading control. **E** Histological scores acquired from HE staining are presented in the methods section. $n=6$ in each group; data are presented as the mean ± SD. $^*P<0.05$ versus the control group. **F** Relative p-IGF-1R densitometry units (OD) from immunohistochemical staining are presented. $n=6$ in each group; data are presented as the mean ± SD; $^*P<0.05$ versus the control group. **G** Serum amylase levels in the indicated pancreas sections of WT mice after caerulein challenge were measured. $n=6$ in each group; all values are presented as the mean ± SD; $^*P<0.05$ versus the control group. **H** Estimations of MPO activity in the indicated pancreas sections from WT mice are presented. $n=6$ in each group; all values are presented as the mean ± SD; $^*P<0.05$ versus the control group

administration (Fig. 2D). These results were consistent with the previous histological changes. Concurrently, as a hallmark of an inflammatory disease, during the acute pancreatitis process, infiltrating leukocytes produce a plethora of pro- and anti-inflammatory cytokines, including TNF-α, IL-1β, IL-8, IL-10, INF-γ, KC, and CXCL2, which exacerbate or mitigate the severity of acute pancreatitis. During the administration of additional drugs, the levels of *TNF-α*, *IL-1β*, *IL-6* and *IL-10* in the pancreases of different groups were detected by real-time PCR. The levels of the proinflammatory cytokines *TNF-α*, *IL-1β*, and *IL-6* were also quantified. Compared with those in the control group, the levels of IL-6 in the acute pancreatitis group, which received picropodophyllotoxin injections, were markedly elevated. The anti-inflammatory cytokine IL-10 exhibited a completely opposite trend, although it was also induced during acute pancreatitis, similar to other cytokines. Furthermore, additional IGF-1 treatment reversed these effects, decreasing the levels of harmful cytokines while increasing the levels of beneficial factors (Fig. 2E, F and G, H). In conclusion, these results suggest that IGF-1 has a protective effect against pancreatitis and that inhibition of IGF-1R aggravates caerulein-induced acute pancreatitis.

β-arrestin1 is involved in IGF-1/IGF-1R-mediated protection in caerulein-induced acute pancreatitis

Although our results demonstrated that IGF-1 is essential for protection against caerulein-induced acute pancreatitis and that additional IGF-1 treatment can attenuate the severity of pancreatitis, the pathway through which IGF-1/IGF-1R induces the protective effect of the pancreas during acute pancreatitis remained unclear. Given that we demonstrated that β-arrestin1 plays an important protective role in caerulein-induced acute pancreatitis, we hypothesized that β-arrestin1 could also be involved in IGF-1/IGF-1R-mediated protection. Western blotting revealed that IGF-1 treatment increased the expression of β-arrestin1, whereas picropodophyllotoxin treatment suppressed it (Fig. 3A). Activation of the transcription factor NF-κB is detectable very early in the course of experimental pancreatitis, and NF-κB is a potent factor in the regulation of inflammation because of its ability to control the expression of numerous inflammatory mediators; therefore, in this study, we also demonstrated that caerulein significantly induced the activation of p65 (p-p65), one of the most important subunits of NF-κB, and that picropodophyllotoxin exacerbated this activity, whereas IGF-1 suppressed it. As a proven suppressor of NF-κB, p-STAT3 tended to reverse the effect of p-p65, which suggested that STAT3 activation could also be involved in the protective function of IGF-1 treatment as a negative regulator of p65 (Fig. 3A). As we mentioned above, β-arrestin1 could also be involved in IGF-1/IGF-1R-mediated protection; thus, we further explored whether this situation could be overcome by targeted deletion of *β-arrestin1* in mice. Interestingly, although we demonstrated that targeted deletion of *β-arrestin1* could aggravate acute pancreatitis induced by caerulein, we also found that *β-arrestin1* deficiency almost completely abolished the protective effect of IGF-1 treatment, as shown by histopathological and histological analyses (Fig. 3B, C). Measurements of amylase activity and MPO activity revealed similar results, further suggesting that β-arrestin1 plays an essential role in the IGF-1/IGF-1R-mediated protection against pancreatitis (Fig. 3D, E). Real-time PCR revealed that IGF-1 administration significantly decreased the levels of *TNF-α*, *IL-1β*, *IL-6* and *IL-10*, whereas the targeted deletion of *β-arrestin1* abolished the effects of IGF-1 treatment (Fig. 3F-I). In summary, *β-arrestin1* knockdown not only aggravated acinar cell injury and inflammatory reactions induced by caerulein but also abrogated IGF-1/IGF-1R-mediated protection, providing evidence that β-arrestin1 is a critical mediator of IGF-1/IGF-1R-mediated protection in caerulein-induced acute pancreatitis.

IGF-1/IGF-1R alleviated acute pancreatitis by suppressing NF-κB through STAT3

The results demonstrated that β-arrestin1 was a critical mediator of IGF-1/IGF-1R-mediated protection against caerulein-induced acute pancreatitis, with p-p65 and p-STAT3 also involved in this process. Western blotting revealed that compared with WT, IGF-1 induced β-arrestin1 expression,

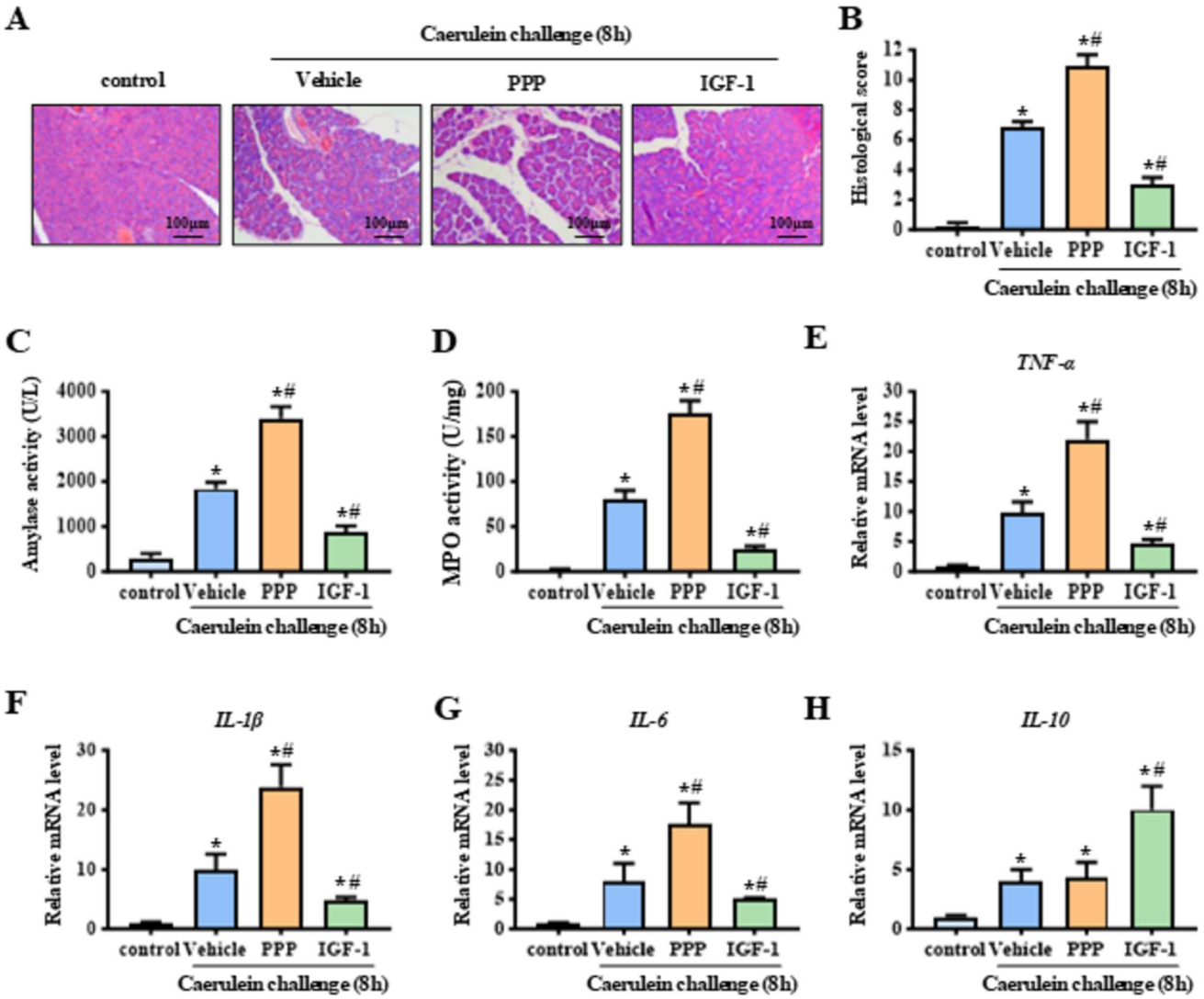


Fig. 2 The inhibition of IGF-1R aggravated caerulein-induced acute pancreatitis. **A** HE staining of the pancreases of WT mice after the indicated treatments is shown (200×). **B** Histological scores acquired from HE staining are presented in the methods section. $n=6$ in each group; data are presented as the mean ± SD; *$P<0.05$ versus the control group; #$P<0.05$ versus the vehicle group. **C** Serum amylase levels in the indicated pancreas sections of WT mice are shown. $n=6$ in each group; all values represent the mean ± SD; *$P<0.05$ versus the control group; #$P<0.05$ versus the vehicle group. **D** Estimation of MPO activity in the indicated pancreas sections from WT mice after the indicated treatments is presented. $n=6$ in each group; all values are presented as the mean ± SD; *$P<0.05$ versus the control group; #$P<0.05$ versus the vehicle group. **E**-**H** The expression levels of the indicated inflammatory factors (*TNF-α*, *IL-1β*, *IL-6* and *IL-10*) in the pancreas of WT mice after the indicated treatments were evaluated by real-time PCR. $n=6$ in each group; data are presented as the mean ± SD; *$P<0.05$ versus the control group; #$P<0.05$ versus the vehicle group

and targeted deletion of β-arrestin1 resulted in a notable decrease in p-STAT3 levels. Concurrently, p-p65 was significantly upregulated, indicating that these components may interact to mediate IGF-1/IGF-1R-mediated protection (Fig. 4A). To observe this interaction, we introduced the STAT3 inhibitor VI S3I-201 (S3I-201, 100 μmol/L). S3I-201 markedly inhibited STAT3 activation without affecting β-arrestin1 expression. Concurrently, p65 activation was significantly enhanced (Fig. 4B). Moreover, S3I-201 exacerbated pancreatic tissue injury and inflammatory responses in WT and *β-arrestin1*-KO mice with pancreatitis that were administered IGF-1 (Fig. 4C and D). The administration of S3I-201 to WT and *β-arrestin*1-KO pancreatitis mice resulted in a significant increase in pancreatic MPO activity compared with that in the respective vehicle group (saline alone). This finding is consistent with the elevated serum amylase levels observed in the same group (Fig. 4E, F). Collectively, these findings indicate that β-arrestin1 may represent an upstream component of the STAT3 pathway, which is induced by IGF-1/IGF-1R. The real-time PCR results demonstrated that the levels of proinflammatory cytokines, including TNF-α, IL-1β, and IL-6, increased significantly

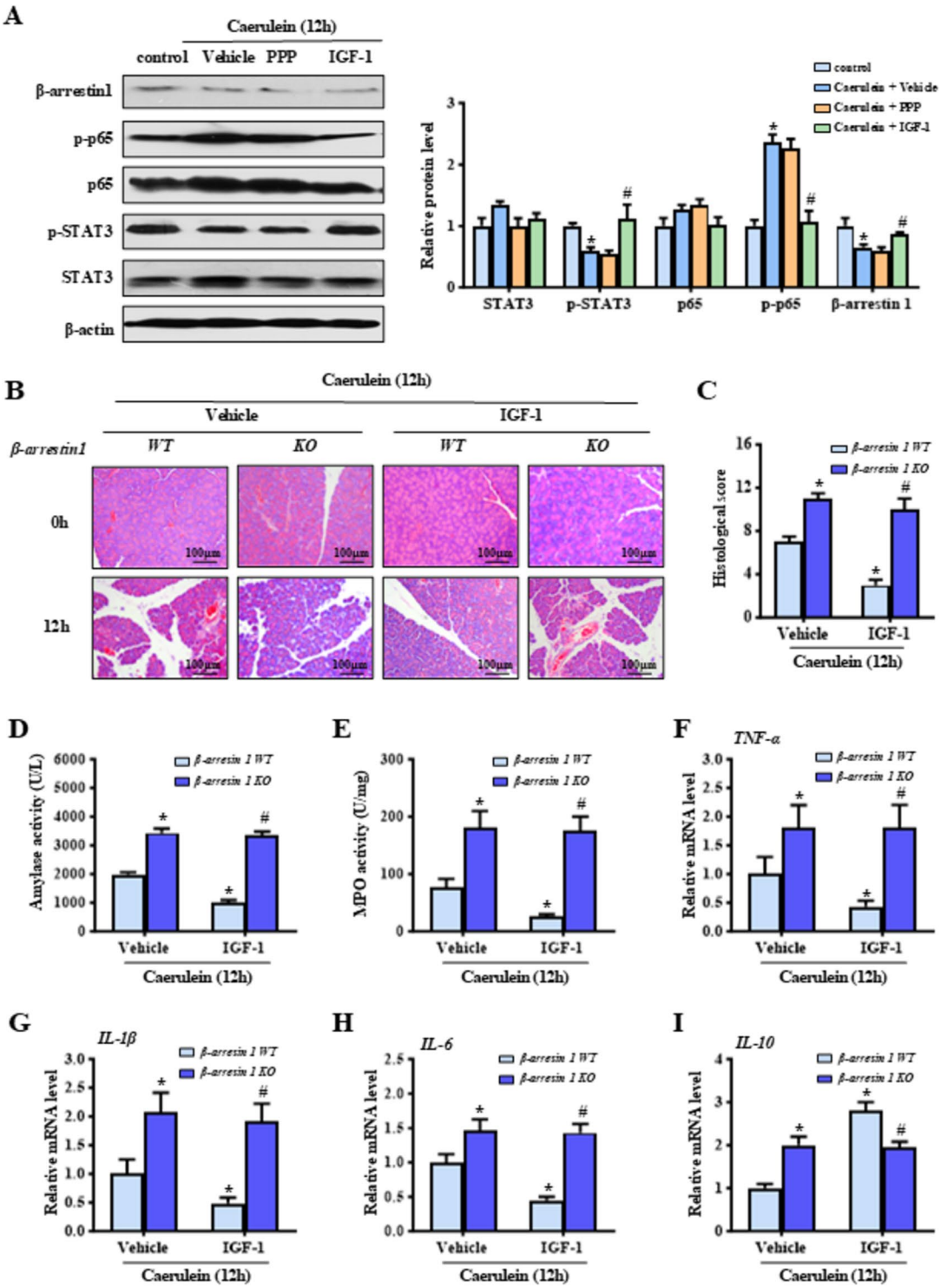
following the blockade of STAT3 activation. Conversely, the levels of the anti-inflammatory cytokine IL-10 decreased significantly. The results demonstrated that IGF-1/IGF-1R alleviated acute pancreatitis by suppressing NF-κB through the inhibition of STAT3, which regulated the inflammatory response by suppressing anti-inflammatory cytokines and inducing proinflammatory cytokines, thereby influencing the process of acute pancreatitis. The results demonstrated that IGF-1/IGF-1R alleviated acute pancreatitis by suppressing NF-κB through STAT3. Conversely, inhibition of STAT3 exacerbated tissue injury and the inflammatory response by upregulating NF-κB activity without influencing the expression of β-arrestin1.

The inhibition of NF-κB enhances IGF-1/IGF-1R-mediated protection in acute pancreatitis

The signaling pathway that mediates the role of IGF-1/IGF-1R in regulating leukocyte infiltration and cytokine production during the process of acute pancreatitis has been the subject of considerable interest. One crucial element, NF-κB, was identified as a powerful regulator of the expression of a range of inflammatory mediators within the pancreas. The available evidence suggests that NF-κB plays a significant role in the early stages of acute pancreatitis and that its inhibition could reduce disease severity [19, 33]. Our data also indicated that the administration of Bay11708 (BAY), an inhibitor of NF-κB, significantly attenuated the severity and inflammatory response in the context of the IGF-1/IGF-1R-mediated protective effect. Western blotting demonstrated that the blockade of NF-κB had no effect on the expression of β-arrestin1 or the activation of STAT3 (Fig. 5A). Pathological analysis demonstrated that the blockade of NF-κB resulted in a reduction in the severity of acute pancreatitis, as evidenced by a decrease in inflammatory cell infiltration, edema and necrosis. These findings were accompanied by comparable amylase levels and histological scores (Fig. 5B, C). In accordance with the findings of other researchers, an examination of MPO activity revealed that blocking NF-κB resulted in a notable reduction in the aggregation of neutrophils in the pancreas, not only in WT mice but also in *β-arrestin1*-KO mice (Fig. 5D, E). The relative levels of inflammatory mediators were quantified to assess the effect of NF-κB blockade on cytokine production. The results demonstrated a significant suppression of proinflammatory cytokines, including TNF-α, IL-1β, and IL-6, while the expression of IL-10 clearly exhibited an upregulation. These findings were observed in the presence of IGF-1 treatment (Supplemental Figs. 2A–D). These results highlight the pivotal role of NF-κB in regulating cytokine production during acute pancreatitis. These findings further suggest that NF-κB blockade not only attenuates the severity of acute pancreatitis but also enhances IGF-1/IGF-1R-mediated protection in acute pancreatitis.

IGF-1/IGF-1R-mediated protection in vitro

To further elucidate the above findings, AR42J cells were treated with caerulein at different time points and concentrations (0, 10, 20, 40, 80, and 100 nmol/L), as well as at different time points (0, 2, 4, 8, 12, and 24 h). Western blotting revealed that both β-arrestin1 expression and p-STAT3 expression were markedly reduced in a time- and concentration-dependent manner following caerulein treatment (Fig. 6A, B). Conversely, p-p65 expression significantly increased during this process. The addition of IGF-1 (100 ng/ml; Sigma) 30 min prior to caerulein (80 nmol/L for 8 h) treatment increased the expression of β-arrestin1 and p-STAT3 but decreased the expression of p-p65. Notably, this treatment did not affect total p65 or STAT3 protein levels (Fig. 6C, Supplemental Fig. 4A). Following the aforementioned results, a β-arrestin1 siRNA oligo kit was used in accordance with the manufacturer's instructions. Subsequently, 100 ng/ml IGF-1 was administered 30 min prior to caerulein (80 nmol/L for a period of eight hours), after which *β-arrestin1* knockdown markedly affected the expression of p-p65 and p-STAT3, with p-p65 upregulation and p-STAT3 downregulation (Fig. 6D, Supplemental Fig. 4B). These findings demonstrate that IGF-1 can mediate the activation of p65 and STAT3 via β-arrestin1. However, the inhibition of STAT3 activation by the STAT3 inhibitor VI S3I-201 (S3I-201, 100 μmol/L) significantly increased p65 activity without affecting the level of β-arrestin1. Additionally, CCK8 experiments confirmed that 100 μmol/L S3I-201 had no significant effect on cell viability (Supplemental Fig. 3), indicating that the observed effects were indeed due to the inhibition of STAT3 rather than cytotoxicity. These findings further proved that STAT3 was the downstream element during the indicated treatment (Fig. 6E, Supplemental Fig. 4C). To further examine the role of NF-κB in this process, the NF-κB inhibitor Bay11708 (BAY, 20 μmol/L) was used. The results revealed that blocking NF-κB activity did not affect the expression of β-arrestin1 or the upregulation of p-STAT3 (Fig. 6F, Supplemental Fig. 4D). These findings are consistent with those of the caerulein-induced pancreatitis model. These observations collectively indicated that β-arrestin1 played a role in the protective effect of caerulein treatment afforded by IGF-1/IGF-1R activity via STAT3 activation and p65 suppression. During this process, STAT3 was identified as an upstream mediator that can regulate p65 activation.



Discussion

Acute pancreatitis is a prevalent inflammatory condition affecting the pancreas and has a spectrum of severity, from mild to severe. The severe form is invariably fatal, with multiorgan failure being an inevitable consequence [34–36]. The severity of acute pancreatitis is undoubtedly attributable to immunological processes that result in injury to acinar cells [19, 37]. This process encompasses the activation and recruitment of inflammatory cells, the local and systemic production and release of inflammatory cytokines, and the final transmigration of activated inflammatory cells across the acinar cell barrier, which exacerbates pancreatic damage [4, 5, 38]. Experimental evidence has shown

Fig. 3 β-arrestin1 is involved in IGF-1/IGF-1R-mediated protection against caerulein-induced acute pancreatitis. **A**β-arrestin1, p-p65, p65, p-STAT3 and STAT3 protein expression levels in the pancreas were determined by Western blotting after the indicated treatments. β-actin was used as the loading control; *$P<0.05$ versus the control group; #$P<0.05$ versus the vehicle group. B AP was induced in *β-arrestin1* knockout and wild-type (WT) mice by 10-h i.p. injections of caerulein. The animals were injected with 80 μg/kg IGF-1 or vehicle 30 min prior to caerulein treatment and sacrificed for analysis 12 h after the last caerulein injection. HE staining of the pancreas from WT and *β-arrestin1*-KO mice after the indicated treatments is shown (200×). C The histological scores acquired from HE staining are presented in the methods section. $n=6$ in each group; data are presented as the mean ± SD; *$P<0.05$ versus the vehicle group of WT mice; #$P<0.05$ versus the IGF-1 group of *β-arrestin1*-KO mice. D Serum amylase levels in the indicated pancreas sections of WT mice and *β-arrestin1*-KO mice are shown. $n=6$ in each group; all values are presented as the mean ± SD; *$P<0.05$ versus the vehicle group of WT mice; #$P<0.05$ versus the IGF-1 group of *β-arrestin1*-KO mice. E MPO activity in the indicated pancreas sections of WT mice and *β-arrestin1*-KO mice is shown. $n=6$ in each group; all values are presented as the mean ± SD; *$P<0.05$ versus the vehicle group of WT mice; #$P<0.05$ versus the IGF-1 group of *β-arrestin1*-KO mice. F-(I) The expression levels of the indicated inflammatory factors (*TNF-α*, *IL-1β*, *IL-6* and *L-10*) in WT mice and *β-arrestin1*-KO mice after the indicated treatments were evaluated by real-time PCR. n = 6 in each group; the data are presented as the mean ± SD; *$P<0.05$ versus the vehicle group of WT mice; #$P<0.05$ versus the IGF-1 group of *β-arrestin1*-KO mice

that the upregulation of inflammatory mediators, including cytokines, chemokines, and inducible nitric oxide, plays a pivotal role in the pathological process of acute pancreatitis [39, 40]. Despite the growing body of evidence indicating potential underlying mechanisms, the pathogenesis of acute pancreatitis remains unclear.

In this study, the administration of caerulein resulted in the development of acute pancreatitis, as evidenced by the elevation of serum amylase levels, the increase in MPO activity, and the observation of distinct pathological alterations at various time points. Previous studies have demonstrated that insulin-like growth factor 1 (IGF-1) is upregulated during experimental acute pancreatitis and may exert a beneficial effect on the pancreas during this process [18]. Moreover, our findings indicated that the mRNA levels of pancreatic IGF-1 were elevated in accordance with the duration of stimulation. Concomitantly, there was an increase in insulin-like growth factor 1 receptor (IGF-1R) autophosphorylation, which was not accompanied by a corresponding change in total IGF-1R expression. Despite the verification of the insulin-like metabolic activity and regulatory role of IGF-1/IGF-1R in cell proliferation, differentiation and apoptosis in a range of cell and tissue types [41–43], autologous IGF-1 has been shown to lack protective efficacy against acinar cell injury in the context of pancreatitis. Notably, subsequent experiments demonstrated that additional IGF-1 treatment significantly attenuated the severity of acute pancreatitis. This was achieved by the suppression of proinflammatory cytokines, including TNF-α, IL-1β, and IL-6, while the upregulation of anti-inflammatory mediators, such as IL-10, was observed. These changes resulted in a reduction in serum amylase levels and a decrease in tissue myeloperoxidase (MPO) activity. In contrast, the administration of an IGF-1R inhibitor, which has the potential to block the autophosphorylation of IGF-1R, unexpectedly exacerbated caerulein-induced pancreatic injury. In our previous research, we demonstrated that β-arrestin1 plays a role in caerulein-induced acute pancreatitis. Moreover, the targeted deletion of β-arrestin1 exacerbates the severity of inflammation in the pancreas by regulating the inflammatory response. β-arrestin1 functions as a multiprotein scaffold, coordinating complex signal transduction networks in diverse cellular processes, including differentiation, cytokine production, proliferation, cell viability, and migration[44–46]. In the present study, a dose-dependent reduction in β-arrestin1 protein expression was observed with increasing severity of pancreatitis. Conversely, β-arrestin1 protein expression markedly increased following IGF-1 treatment. These findings indicate that β-arrestin1 may be involved in the protective effects of IGF-1 in acute pancreatitis. However, the precise targets and underlying mechanism of pancreatic protection remain incompletely understood.

The recruitment of β-arrestin1 to the IGF-1 receptor (IGF-1R) upon binding to IGF-1 has been demonstrated, and this protein is capable of regulating downstream signaling networks, thereby influencing the function of these cells. Similarly, β-arrestin1-mediated IGF-1 signaling, like the IGF-1 signaling pathway, activates the PI3K/AKT, MAPK and other pathways, thereby promoting cell differentiation, proliferation and other cellular processes [47–49]. On the basis of these findings, *β-arrestin1*-WT mice and *β-arrestin1*-KO mice were treated with caerulein. *β-arrestin1* deficiency resulted not only in the deterioration of acute pancreatitis but also in the abolition of the protective effect of IGF-1 treatment and serum amylase under similar conditions. Compared with that in their wild-type (WT) counterparts, the activity of myeloperoxidase (MPO), which is a marker of neutrophil sequestration within the organ, was markedly elevated in *β-arrestin1*-KO pancreatitis mice under IGF-1 treatment, accompanied by the expression of tumor necrosis factor alpha (TNF-α), interleukin 1 beta (IL-1β) and interleukin 6 (IL-6), which promote an inflammatory response. In contrast, the anti-inflammatory mediator IL-10, which can reduce macrophage activation and inhibit the production of reactive oxygen species, was suppressed in *β-arrestin1*-KO pancreatitis mice that were administered IGF-1. On the basis of these findings, examining how β-arrestin1 regulates the aforementioned events in acinar cells would be interesting.

One crucial signaling molecule, NF-κB, plays a pivotal role in the etiology of acute pancreatitis. As a nuclear

transcription factor, NF-κB is responsible for regulating the transcription of a wide variety of genes involved in immunity and inflammation [50, 51]**.** A substantial body of evidence highlights the pivotal role of these genes in the pathogenesis of acute pancreatitis. In the present study, *β-arrestin1* deficiency increased the main components of NF-κB, p65 activation, in comparison to their WT counterparts, regardless of IGF-1 treatment in the pancreatitis model. These findings prompted us to investigate the mechanisms by which β-arrestin1 regulates NF-κB activity. Additionally, we observed that STAT3 activation occurred during IGF-1 treatment in WT pancreatitis models, a phenomenon that was not evident in *β-arrestin1*-KO mice. The specific inhibition of STAT3 markedly suppressed p65 activation during the course of the experiment, thereby preventing the protective effects of IGF-1 in acute pancreatitis. Notably, the STAT3 inhibitor did not affect the status of β-arrestin1, despite markedly exacerbating pancreatic injury and the inflammatory response throughout the process. Furthermore, the administration of an NF-κB inhibitor resulted in a significant reduction in the severity of acute pancreatitis, the inflammatory response, and cytokine levels. Furthermore, it successfully reversed the detrimental effects of β-arrestin1-mediated tissue damage without affecting the status of β-arrestin1 and STAT3 activation. In vitro experiments utilizing the AR42J cell line provided further validation of this signaling network during IGF-1-mediated protection from caerulein stimulation. Importantly, these findings indicated that β-arrestin1 suppressed NF-κB activation through a STAT3-mediated mechanism in the protective effect of IGF-1 in caerulein-induced pancreatitis.

Nonetheless, our co-immunoprecipitation assays did not detect a direct interaction between STAT3 and IGF-1R, consistent with prevailing knowledge in the field. While β-arrestin1 is well established as a scaffold protein for IGF-1R—promoting Mdm2-dependent ubiquitination as well as activation of ERK and PI3K pathways—current literature does not substantiate a direct association between β-arrestin1 and STAT3, nor the recruitment of STAT3 to IGF-1R. Our experimental data corroborate the absence of direct binding between STAT3 and IGF-1R, aligning with the recognized interaction profile of β-arrestin1 involving partners such as Mdm2, Src, and PI3K.This indicates that the activation of STAT3 by IGF-1R signaling may be mediated through other mechanisms or intermediates, rather than through a direct physical interaction with β-arrestin1. Additional research is required to clarify the exact mechanisms involved, which we intend to examine in subsequent studies.

The interplay between STAT3 and NF-κB is characterized by a multifaceted network involving both direct and indirect interactions. STAT3 has been shown to directly associate with components of NF-κB, thereby influencing their nuclear translocation and functional activity. Under certain conditions, STAT3 can also directly inhibit NF-κB-mediated transcriptional activation [52, 53]. Additionally, STAT3 modulates NF-κB signaling indirectly through upstream regulators, such as microRNA-mediated pathways, and via crosstalk with other signaling cascades, including the PI3K pathway [54, 55]. In the present study, we demonstrate that IGF-1/IGF-1R signaling confers protective effects in acute pancreatitis by regulating both STAT3 and NF-κB pathways. The observed inverse correlation between phosphorylated STAT3 (p-STAT3) and phosphorylated p65 (p-p65) levels implies that activation of STAT3 may contribute to the suppression of NF-κB activity. Nevertheless, the precise molecular mechanisms governing this interaction within the context of acute pancreatitis remain to be elucidated. Future investigations should aim to delineate the specific pathways mediating STAT3 and NF-κB crosstalk and to identify potential therapeutic targets capable of modulating this interaction.

In conclusion, IGF-1 may protect the pancreas from damage and the inflammatory response induced by caerulein through the upregulation of β-arrestin1 expression during the acute phase of pancreatitis. β-arrestin1 markedly attenuates the severity of this disease and the degree of inflammation by activating the STAT3 pathway, which may additionally inhibit NF-κB activation. These results indicate that β-arrestin1 plays a role in mediating the protective effects of IGF-1 in caerulein-induced acute pancreatitis. Thus, the IGF-1/IGF-1R/β-arrestin1 signaling pathway may serve as a prospective therapeutic target for acute pancreatitis.

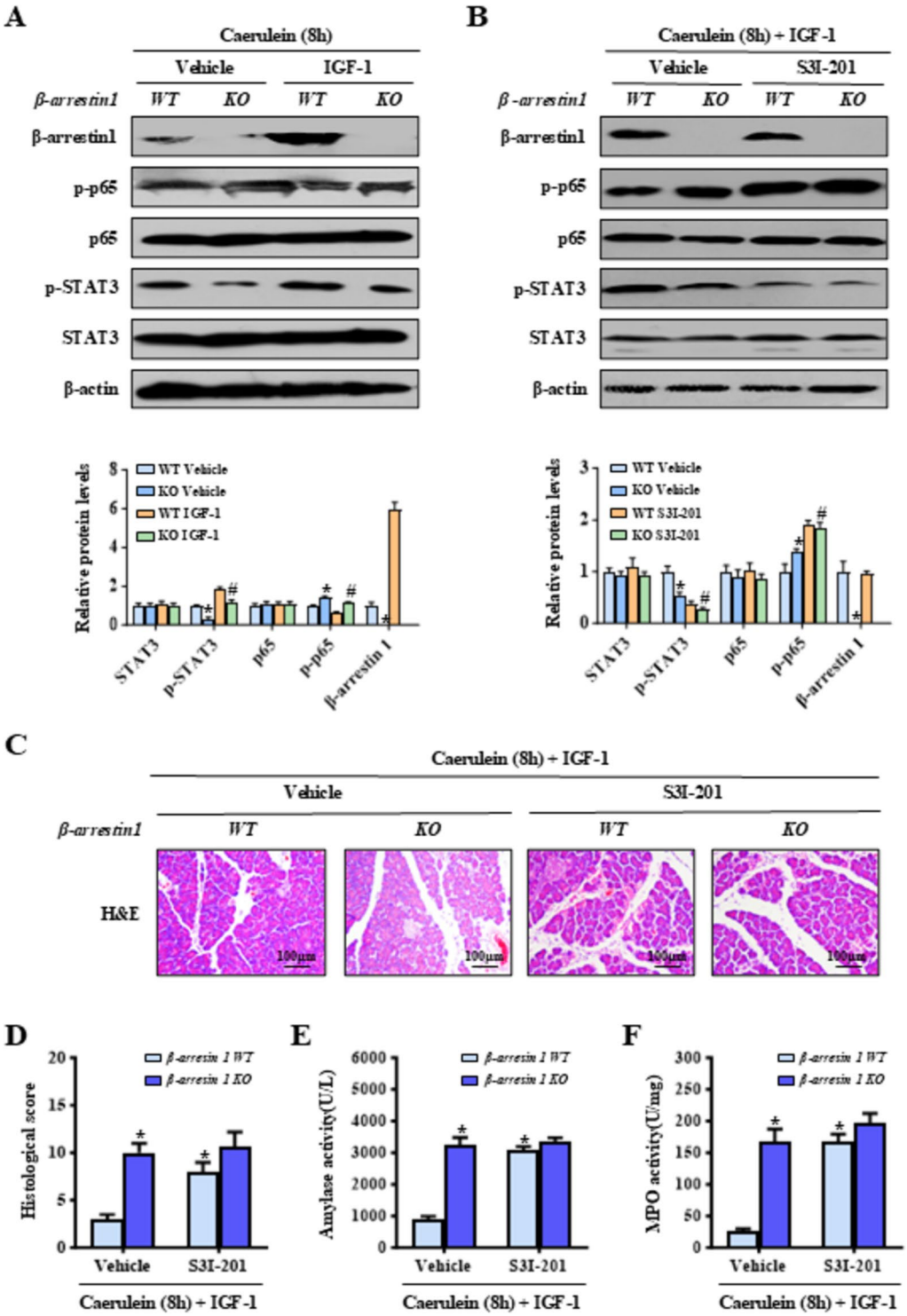


Fig. 4 IGF-1/IGF-1R alleviated acute pancreatitis by suppressing NF-κB through STAT3. **A** Western blotting for β-arrestin1, p-p65, p65, p-STAT3 and STAT3 in the pancreases of WT and *β-arrestin1*-KO mice after the indicated treatments. β-actin served as a loading control; **P* < 0.05 versus the vehicle group from WT mice; #*P* < 0.05 versus the vehicle group from *β-arrestin1*-KO mice. **B** p-p65, p65, p-STAT3 and STAT3 protein expression in the pancreas was determined by Western blotting after the indicated treatments. β-actin was used as the loading control. (**C**) HE staining of the pancreas of WT and *β-arrestin1*-KO mice after the indicated treatments is shown (200×). **D** Histological scores summarized from HE staining are shown. n = 6 in each group; the data are presented as the mean ± SD; **P* < 0.05 versus the vehicle group of WT mice. **E** Serum amylase levels in the indicated pancreas sections of WT mice and *β-arrestin1*-KO mice are shown. n = 6 in each group; all values are presented as the mean ± SD; **P* < 0.05 versus the vehicle group of WT mice. **F** MPO activity in the indicated pancreas sections of WT mice and *β-arrestin1*-KO mice was determined. n = 6 in each group; all values are presented as the mean ± SD; **P* < 0.05 versus the vehicle group of WT mice

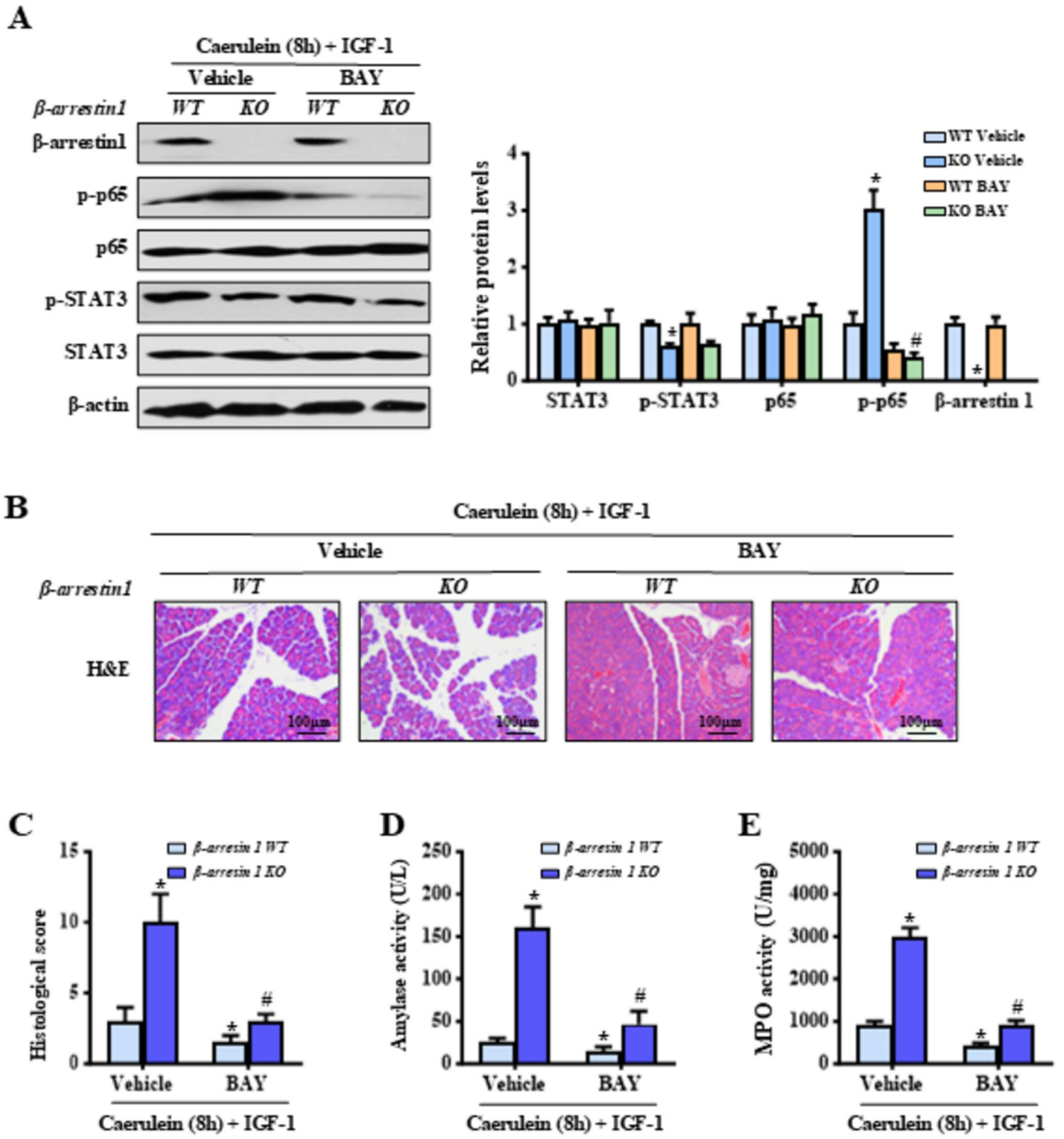


Fig. 5 Inhibition of NF-κB enhances IGF-1/IGF-1R-mediated protection in acute pancreatitis. **A** P-p65, p65, p-STAT3 and STAT3 levels in the pancreases of WT and *β-arrestin1*-KO mice after the indicated treatments were detected by Western blotting. β-actin served as a loading control; **P*<0.05 versus the vehicle group from WT mice; #*P*<0.05 versus the vehicle group from *β-arrestin1*-KO mice. **B** Pancreatitis was induced by caerulein or 80 μg/kg IGF-1, and BAY or vehicle was administered once 30 min before the first caerulein injection.Serum amylase levels in the indicated pancreas sections of WT mice and *β-arrestin1*-KO mice were evaluated. n=6 in each group; all data are presented as the mean±SD; **P*<0.05 versus the vehicle group of WT mice; #*P*<0.05 versus the vehicle group of *β-arrestin1*-KO mice. HE staining of the pancreas of WT and *β-arrestin1*-KO mice after the indicated treatments is shown (200×). C Histological scores evaluated by HE staining are shown. n=6 in each group; data are presented as the mean±SD; **P*<0.05 versus the vehicle group of WT mice; #*P*<0.05 versus the vehicle group of *β-arrestin1*-KO mice. D Serum amylase levels in the indicated pancreas sections of WT mice and *β-arrestin1-KO* mice is shown. n=6 in each group; all values are presented as the mean±SD; **P*<0.05 versus the vehicle group of WT mice; #*P*<0.05 versus the vehicle group of *β-arrestin1*-KO mice. E The determination of MPO activity in the indicated pancreas sections of WT mice and *β-arrestin1-KO* mice is shown. n=6 in each group; all values are presented as the mean±SD; **P*<0.05 versus the vehicle group of WT mice; #*P*<0.05 versus the vehicle group of *β-arrestin1*-KO mice

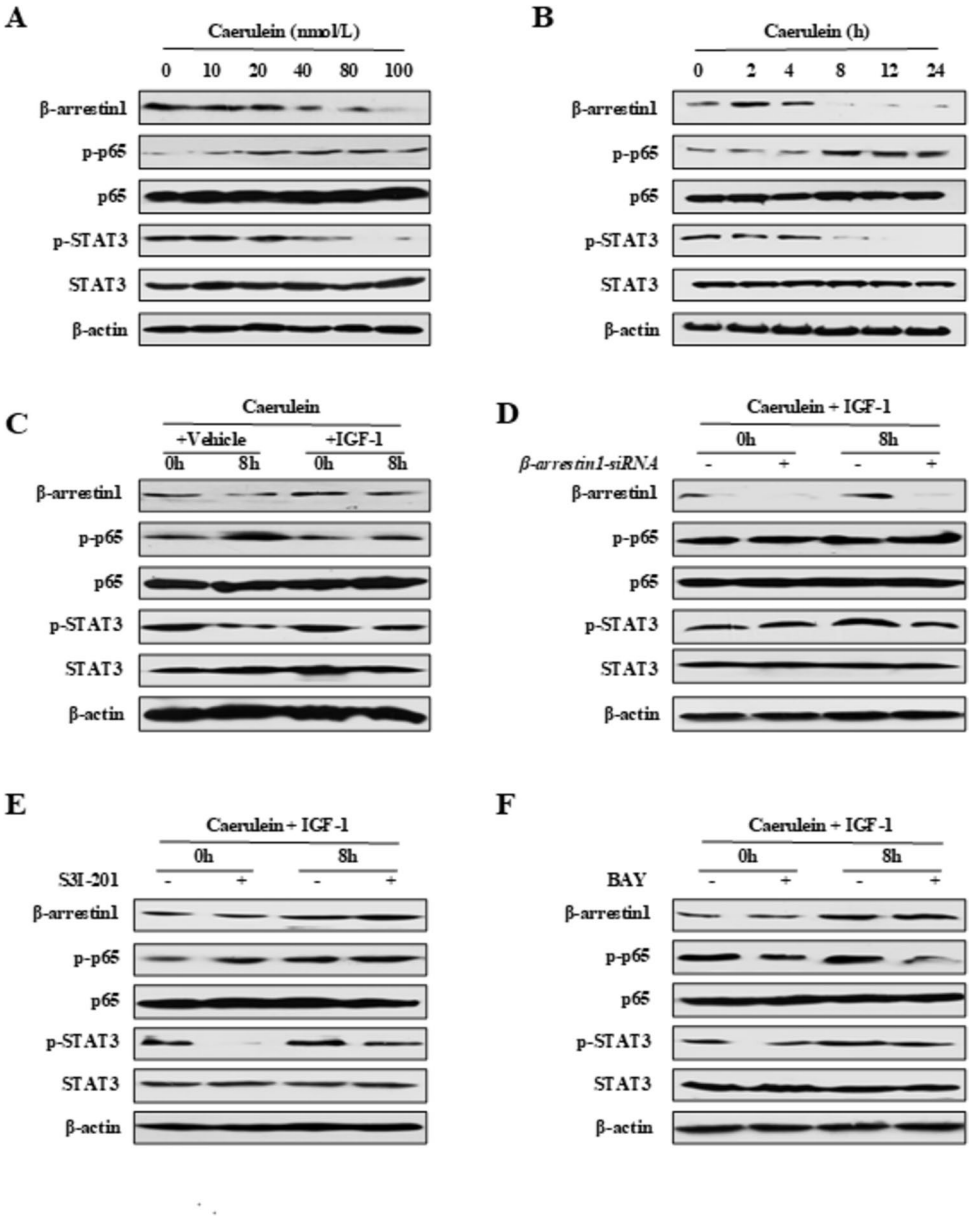


Fig. 6 IGF-1/IGF-1R-mediated protection in vitro. **A** AR42J cells were treated with caerulein at the indicated concentrations for 12 h, and Western blotting revealed that the expression of β-arrestin1, p-p65, and p-STAT3 significantly changed in response to stimulation with different concentrations of caerulein, whereas the expression levels of p65 and STAT3 were not affected. β-actin was used as the loading control. **B** AR42J cells were treated with 80 nmol/L caerulein for the indicated time intervals, and Western blotting analysis revealed that the expression levels of β-arrestin1, p-p65, and p-STAT3 and were significantly altered upon stimulation with caerulein, whereas the expression levels of p65 and STAT3 were not affected. β-actin was used as the loading control. **C** IGF-1 (100 ng/ml) was given 30 min prior to caerulein (80 nmol/L for 8 h) treatment, and β-arrestin1, p65, p-p65, STAT3 and p-STAT3 protein expression was detected by Western blotting. β-actin was used as the loading control. **D** After incubation for 24 h, the transfection medium was replaced with regular culture medium before the indicated treatment (IGF-1 (100 ng/ml) given 30 min prior to caerulein (80 nmol/L for 8 h)). β-arrestin1, p65, p-p65, STAT3 and p-STAT3 protein expression was examined by Western blotting. β-actin was used as the loading control. **E** AR42J cells were stimulated with the indicated treatment (IGF-1 (100 ng/ml) given 30 min prior to caerulein (80 nmol/L for 8 h) in the presence or absence of the STAT3 inhibitor VI S3I-201. The lysates were subjected to Western blotting analysis for β-arrestin1, p65, p-p65, STAT3 and p-STAT3 protein expression. β-actin was used as the loading control. Cell viability was assessed using the CCK-8 assay as described in the methods. **F** AR42J cells were stimulated with the indicated treatment (IGF-1 (100 ng/ml) given 30 min prior to caerulein (80 nmol/L for 8 h) in the presence or absence of the NF-κB inhibitor Bay11708 (BAY, 20 μmol/L), and the results of Western blotting analysis for β-arrestin1, p65, p-p65, STAT3 and p-STAT3 protein expression are presented. β-actin was used as the loading control

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11010-025-05435-4.

Author contributions These authors contributed equally: Xiaoli Huang, Li Tao **Xiaoli Huang**: Conceptualization, Methodology, Investigation, Formal Analysis, Writing – Original Draft **Li Tao**: Investigation, Formal Analysis, Resources, Writing – Review & Editing **Huiling Liu**: Methodology, Validation, Data Curation **Hongju Luo**: Visualization, Data Curation **Yunwei Guo**: Conceptualization, Supervision, Project Administration, Funding Acquisition, Writing – Review & Editing.

Funding The project was supported by the National Natural Science Foundation of China (No. 82370599).

Data availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest The authors declare no competing interests.

References

1. Schepers NJ, Bakker OJ, Besselink MG, Ali UA, Bollen TL, Gooszen HG, van Santvoort HC, Bruno MJ (2019) Impact of characteristics of organ failure and infected necrosis on mortality in necrotising pancreatitis. Gut 68:1044–1051
2. Kruger B, Albrecht E, Lerch MM (2000) The role of intracellular calcium signaling in premature protease activation and the onset of pancreatitis. Am J Pathol 157:43–50
3. Bhatia M, Wong FL, Cao Y, Lau HY, Huang J, Puneet P, Chevali L (2005) Pathophysiology of acute pancreatitis. Pancreatology 5:132–144
4. Dawra R, Sah RP, Dudeja V, Rishi L, Talukdar R, Garg P, Saluja AK (2011) Intra-acinar trypsinogen activation mediates early stages of pancreatic injury but not inflammation in mice with acute pancreatitis. Gastroenterology 141:2210-2217.e2
5. Saluja A, Dudeja V, Dawra R, Sah RP (2019) Early intra-acinar events in pathogenesis of pancreatitis. Gastroenterology 156:1979–1993
6. Barreto SG, Habtezion A, Gukovskaya A, Lugea A, Jeon C, Yadav D, Hegyi P, Venglovecz V, Sutton R, Pandol SJ (2020) Critical thresholds: key to unlocking the door to the prevention and specific treatments for acute pancreatitis. Gut 70:194–203
7. Gukovsky I, Li N, Todoric J, Gukovskaya A, Karin M (2013) Inflammation, autophagy, and obesity: common features in the pathogenesis of pancreatitis and pancreatic cancer. Gastroenterology 144:1199–209.e4
8. Pandol SJ, Saluja AK, Imrie CW, Banks PA (2007) Acute pancreatitis: bench to the bedside. Gastroenterology 132:1127–1151
9. Sah RP, Saluja A (2011) Molecular mechanisms of pancreatic injury. Curr Opin Gastroenterol 27:444–451
10. Makhija R, Kingsnorth AN (2002) Cytokine storm in acute pancreatitis. J Hepatobiliary Pancreat Surg 9:401–410
11. Huat TJ, Khan AA, Pati S, Mustafa Z, Abdullah JM, Jaafar H (2014) IGF-1 enhances cell proliferation and survival during early differentiation of mesenchymal stem cells to neural progenitor-like cells. BMC Neurosci 15:91
12. D'Ercole AJ (1996) Insulin-like growth factors and their receptors in growth. Endocrin Metab Clin 25:573–590
13. Emmerson E, Campbell L, Davies FCJ, Ross NL, Ashcroft GS, Krust A, Chambon P, Hardman MJ (2012) Insulin-like growth factor-1 promotes wound healing in estrogen-deprived mice: new insights into cutaneous IGF-1R/ERalpha cross talk. J Invest Dermatol 132:2838–2848
14. Zheng W, Kar S, Quirion R (2002) Insulin-like growth factor-1-induced phosphorylation of transcription factor FKHRL1 is mediated by phosphatidylinositol 3-kinase/Akt kinase and role of this pathway in insulin-like growth factor-1-induced survival of cultured hippocampal neurons. Mol Pharmacol 62:225–233
15. Gukovskaya AS, Gukovsky I (2012) Autophagy and pancreatitis. Am J Physiol-Gastr Liver Physiol 303:G993–G1003
16. Himpe E, Kooijman R (2009) Insulin-like growth factor-I receptor signal transduction and the Janus kinase/signal transducer and activator of transcription (JAK-STAT) pathway. BioFactors 35:76–81
17. Gao L, Wang X, Wang X, Zhang L, Qiang C, Chang S, Ren W, Li S, Yang Y, Tong D, Chen C, Li Z, Song T, Zhi K, Huang C (2014) IGF-1R, a target of let-7b, mediates crosstalk between IRS-2/Akt and MAPK pathways to promote proliferation of oral squamous cell carcinoma. Oncotarget 5:2562–2574
18. Ceranowicz D, Warzecha Z, Dembinski A, Ceranowicz P, Cieszkowski J, Kusnierz-Cabala B, Tomaszewska R, Kuwahara A, Kato I (2010) Role of hormonal axis, growth hormone - IGF-1, in the therapeutic effect of ghrelin in the course of cerulein-induced acute pancreatitis. J Physiol Pharmacol 61:599–606
19. Gukovsky I, Pandol SJ, Gukovskaya AS (2011) Organellar dysfunction in the pathogenesis of pancreatitis. Antioxid Redox Signal 15:2699–2710
20. Shenoy SK, Lefkowitz RJ (2011) Beta-arrestin-mediated receptor trafficking and signal transduction. Trends Pharmacol Sci 32:521–533
21. Girnita L, Shenoy SK, Sehat B, Vasilcanu R, Vasilcanu D, Girnita A, Lefkowitz RJ, Larsson O (2007) Beta-arrestin and Mdm2 mediate IGF-1 receptor-stimulated ERK activation and cell cycle progression. J Biol Chem 282:11329–11338
22. Drake MT, Violin JD, Whalen EJ, Wisler JW, Shenoy SK, Lefkowitz RJ (2008) Beta-arrestin-biased agonism at the beta2-adrenergic receptor. J Biol Chem 283:5669–5676
23. Shukla AK, Xiao K, Lefkowitz RJ (2011) Emerging paradigms of beta-arrestin-dependent seven transmembrane receptor signaling. Trends Biochem Sci 36:457–469
24. Luttrell LM, Lefkowitz RJ (2002) The role of beta-arrestins in the termination and transduction of G-protein-coupled receptor signals. J Cell Sci 115:455–465
25. Gurevich VV, Gurevich EV (2006) The structural basis of arrestin-mediated regulation of G-protein-coupled receptors. Pharmacol Ther 110:465–502

26. Gurevich EV, Tesmer JJG, Mushegian A, Gurevich VV (2012) G protein-coupled receptor kinases: more than just kinases and not only for GPCRs. Pharmacol Ther 133:40–69
27. Povsic TJ, Kohout TA, Lefkowitz RJ (2003) Beta-arrestin1 mediates insulin-like growth factor 1 (IGF-1) activation of phosphatidylinositol 3-kinase (PI3K) and anti-apoptosis. J Biol Chem 278:51334–51339
28. Peterson YK, Luttrell LM (2017) The diverse roles of Arrestin scaffolds in G protein-coupled receptor signaling. Pharmacol Rev 69:256–297
29. Lee T, Lee E, Irwin R, Lucas PC, McCabe LR, Parameswaran N (2013) Beta-arrestin-1 deficiency protects mice from experimental colitis. Am J Pathol 182:1114–1123
30. Li P, Cook JA, Gilkeson GS, Luttrell LM, Wang L, Borg KT, Halushka PV, Fan H (2011) Increased expression of beta-arrestin 1 and 2 in murine models of rheumatoid arthritis: isoform specific regulation of inflammation. Mol Immunol 49:64–74
31. Tao L, Lin X, Tan S, Lei Y, Liu H, Guo Y, Zheng F, Wu B (2019) β-Arrestin1 alleviates acute pancreatitis via repression of NF-κBp65 activation. J Gastroenterol Hepatol 34:284–292
32. Warzecha Z, Dembinski A, Ceranowicz P, Konturek SJ, Tomaszewska R, Stachura J, Konturek PC (2003) IGF-1 stimulates production of interleukin-10 and inhibits development of caerulein-induced pancreatitis. J Physiol Pharmacol 54:575–590
33. Algul H, Treiber M, Lesina M, Nakhai H, Saur D, Geisler F, Pfeifer A, Paxian S, Schmid RM (2007) Pancreas-specific RelA/p65 truncation increases susceptibility of acini to inflammation-associated cell death following cerulein pancreatitis. J Clin Invest 117:1490–1501
34. Cook ME, Bruun NH, Davidsen L, Drewes AM, Olesen SS (2023) Multistate model of the natural history of inflammatory pancreatic diseases: a nationwide population-based cohort study. Gastroenterology 165:1547-1557.e4
35. Banks PA, Freeman ML (2006) Practice guidelines in acute pancreatitis. Am J Gastroenterol 101:2379–2400
36. Garg PK, Singh VP (2019) Organ failure due to systemic injury in acute pancreatitis. Gastroenterology 156:2008–2023
37. Stojanovic B, Jovanovic IP, Stojanovic MD, Jovanovic M, Vekic B, Milosevic B, Cvetkovic A, Spasic M, Stojanovic BS (2023) The emerging roles of the adaptive immune response in acute pancreatitis. Cells. https://doi.org/10.3390/cells12111495
38. Sendler M, Weiss F, Golchert J, Homuth G, van den Brandt C, Mahajan UM, Partecke L, Doring P, Gukovsky I, Gukovskaya AS, Wagh PR, Lerch MM, Mayerle J (2018) Cathepsin B-mediated activation of trypsinogen in endocytosing macrophages increases severity of pancreatitis in mice. Gastroenterology 154:704-718.e10
39. Sternby H, Hartman H, Thorlacius H, Regnér S (2021) The initial course of IL1β, IL-6, IL-8, IL-10, IL-12, IFN-γ and TNF-α with regard to severity grade in acute pancreatitis. Biomolecules 11:591
40. Gukovskaya AS, Gukovsky I, Algul H, Habtezion A (2017) Autophagy, inflammation, and immune dysfunction in the pathogenesis of pancreatitis. Gastroenterology 153:1212–1226
41. Mizoguchi A, Okamoto N (2013) Insulin-like and IGF-like peptides in the silkmoth *Bombyx mori*: discovery, structure, secretion, and function. Front Physiol 4:217
42. Wolfe A, Divall S, Wu S (2014) The regulation of reproductive neuroendocrine function by insulin and insulin-like growth factor-1 (IGF-1). Front Neuroendocrinol 35:558–572
43. LeRoith D, Roberts CTJ (2003) The insulin-like growth factor system and cancer. Cancer Lett 195:127–137
44. Grady EF (2007) Cell signaling Beta-arrestin, a two-fisted terminator. Science 315:605–606
45. Kahsai AW, Shah KS, Shim PJ, Lee MA, Shreiber BN, Schwalb AM, Zhang X, Kwon HY, Huang L, Soderblom EJ, Ahn S, Lefkowitz RJ (2023) Signal transduction at GPCRs: allosteric activation of the ERK MAPK by β-arrestin. Proc Natl Acad Sci U S A. https://doi.org/10.1073/pnas.2303794120
46. Kahsai AW, Shah KS, Shim PJ, Lee MA, Shreiber BN, Schwalb AM, Zhang X, Kwon HY, Huang L, Soderblom EJ, Ahn S, Lefkowitz RJ (2023) Signal transduction at GPCRs: allosteric activation of the ERK MAPK by beta-arrestin. P Natl Acad Sci USA 120:e2303794120
47. Manning BD, Toker A (2017) AKT/PKB signaling: navigating the network. Cell 169:381–405
48. Jajoo S, Mukherjea D, Kumar S, Sheth S, Kaur T, Rybak LP, Ramkumar V (2010) Role of beta-arrestin1/ERK MAP kinase pathway in regulating adenosine A1 receptor desensitization and recovery. Am J Physiol-Cell Ph 298:C56-65
49. Ma L, Pei G (2007) Beta-arrestin signaling and regulation of transcription. J Cell Sci 120:213–218
50. Giuliani C, Napolitano G, Bucci I, Montani V, Monaco F (2001) Nf-kB transcription factor: role in the pathogenesis of inflammatory, autoimmune, and neoplastic diseases and therapy implications. Clin Ter 152:249–253
51. Guo Q, Jin Y, Chen X, Ye X, Shen X, Lin M, Zeng C, Zhou T, Zhang J (2024) NF-kappaB in biology and targeted therapy: new insights and translational implications. Signal Transduct Tar 9:53
52. Lee H, Herrmann A, Deng J, Kujawski M, Niu G, Li Z, Forman S, Jove R, Pardoll DM, Yu H (2009) Persistently activated Stat3 maintains constitutive NF-kappaB activity in tumors. Cancer Cell 15:283–293
53. Lee H, Deng J, Xin H, Liu Y, Pardoll D, Yu H (2011) A requirement of STAT3 DNA binding precludes Th-1 immunostimulatory gene expression by NF-kappaB in tumors. Cancer Res 71:3772–3780
54. Xiang M, Birkbak NJ, Vafaizadeh V, Walker SR, Yeh JE, Liu S, Kroll Y, Boldin M, Taganov K, Groner B, Richardson AL, Frank DA (2014) STAT3 induction of miR-146b forms a feedback loop to inhibit the NF-kappaB to IL-6 signaling axis and STAT3-driven cancer phenotypes. Sci Signal 7:ra11
55. Zhu Y, Gu L, Li Y, Lin X, Shen H, Cui K, Chen L, Zhou F, Zhao Q, Zhang J, Zhong B, Prochownik E, Li Y (2017) Mir-148a inhibits colitis and colitis-associated tumorigenesis in mice. Cell Death Differ 24:2199–2209