Expression and functional characterization of a novel antimicrobial peptide: human beta-defensin118

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Abstract

Background: β-defensin 118 (DEFB118) is a novel host defence peptide (HDP) identified in human. To evaluate its potentials for future utilization, the DEFB118 gene was expressed in *Escherichia coli* (*E. coli*) and the recombinant protein was fully characterized.

Methods: The DEFB118 protein was obtained by heterologous expression using *E. coli* Rosetta (DE3). Antibacterical activity of DEFB118 were determined by using various bacterial strains. IPEC-J cells challenged by *E. coli* K88 were used to determine its influences on inflammatory responses.

Results: The *E. coli* transformants yielded more than 250 mg/mL D EFB118 protein after 4 h induction by 1.0 mM IPTG. The DEFB118 was estimated by SDS-PAGE to be 30 kDa, and MALDI-TOF analysis verified it is a human β-defensin 118. Importantly, the DEFB118 showed antimicrobial activities against both Gram-negative bacteria (*E. coli* K88 and *E. coli* DH5α) and Gram-positive bacteria (S. aureus and B. subtilis), with a minimum inhibitory concentration (MIC) of 4 µg/mL. Hemolytic assays showed that DEFB118 had no detrimental impact on cell viability. Additionally, DEFB118 was found to elevate the viability of IPEC-J2 cells upon *E. coli* K88 challenge. Moreover, DEFB118 significantly decreased cell apoptosis in the late apoptosis phase and down-regulated the expression of inflammatory cytokines such as the IL-1β and TNF-a in the IPEC-J2 cells exposure to *E. coli* K88.

Conclusions: These results suggested a novel function of the mammalian defensins, and the anti-bacterial and anti-inflammatory properties of DEFB118 may allow it a potential substitute for conventionally used antibiotics or drugs.

Background

In the last decades, antibiotics have been widely used as a therapeutic medicine to control various infectious diseases [1−3]. However, their continuous use not only leads to serious environmental pollution, but also increases the risk of developing drug resistance [4−8]. Therefore, developing of novel substitutes for conventionally used antibiotics has attracted considerable research interest worldwide [9−12]. Host defence peptides (HDPs), a major subclass of the antimicrobial peptide (AMP) family, are expressed in a variety of epithelial tissues and cells [13, 14]. Previous studies indicated that HDPs can disrupt the bacterial membranes by forming nonspecific electrostatic interactions with membrane lipids, and therefore can protect host from a broad range of pathogens including bacteria, virus and fungus [15−17]. Considering with the fact that bacteria are less able to develop resistance to HDPs than to traditional antibiotics [17], the administration of HDPs is a potentially novel therapeutic strategy for infectious diseases, and may present a promising alternative to the traditional antibiotics.

Defensins comprise an important family of HDPs for mammalian animals. According to distribution patterns of the intra-molecular disulfide bonds, defensins are classified into α- and β-forms [18, 19]. The α-defensins are expressed in neutrophils and paneth cells, whereas the β-defensins are usually expressed in the epithelia. Recent studies indicated that the β-defensins has multidirectional biological properties,

including antiviral, antibacterial, and anti-inflammatory effects [20, 21]. For instance, β-defensin 114 was found to show antimicrobial activities against E. coli DH5α and E. coli K88 [22]. While, β-defensin 129 was reported to attenuate intestinal inflammation and epithelial atrophy in rat exposure to bacterial endotoxin [14]. The humanβ-defensin 118 (DEFB118) is novel identified HDP, which is present in the epithelial cells of different ducts and most abundant in the caput epithelium, where it is present in the lumen and located on sperm [23]. Previous study indicated that the DEFB118 caused rapid permeabilization of both outer and inner membranes of E. coli and striking morphological alterations in the bacterial surfaces [24]. Therefore, the DEFB118 may contribute to epididymal innate immunity and protect the sperm against attack by microorganisms in the male and female reproductive tracts.

Although, numerous studies indicated a critical role of β-defensins in the host defence against exogenous pathogens, the functions of DEFB118 protein has not been fully characterized. Moreover, the production of DEFB118 is still not commercial feasible because of its high cost. In the present study, we describe the cloning and expression of DEFB118 gene by pET expression vector in E. coli. In addition, the antimicrobial and anti-inflammatory activities of the DEFB118protein were fully characterized.

Materials And Methods

Strains and Vectors

The E. coli DH5α and E. coli Rosetta (DE3) strains were purchased from TIANGEN (China). E. coli K88 was kindly provided by Professor LianqiangChe, Institute of Animal Nutrition, Sichuan Agricultural University. Salmonella typhimurium TCC14028 (S. typhimurium), Streptococcus, Staphylococcus aureus CICC23656 (S. aureus) and Bacillus subtilis (B. subtilis) were kindly provided by Professor Qigui Yan, Collegeof Animal Science and Technology, Sichuan Agricultural University. The pET32a (+) was purchased from Invitrogen.

Plasmid Construction

DEFB118 gene was obtained by complete gene synthesis (Beijing cycle-tech biotechnology co., LTD., China). The company provides a cloned strain of DH5α-PMD19-DEFB118 containing the target gene DEFB118. And sequencing identification in Shenggong biological engineering co., LTD. Sequencing results were compared by software DNAMAN. The plasmid PMD19-DEFB118 was extracted by using Plasmid Mini Kit I (Omega, America) according to the manufacturer's recommendations. The plasmid pMD19-DEFB118 and expression vector pET32a (+) were double-digested with EcoRI and NotI enzymes (Takara, Japan) at 37 °C for 4 h. After purification by agarose gel electrophoresis, the isolated DNA fragments were ligated by T4 DNA ligase (Takara, Japan). And then, the ligated product was transformed into E. coli DH5α cells using the heat shock method and plated on LB agar containing kanamycin (50 μg/mL). The positive colonies were randomly picked, then confirmed by restriction enzyme digestion and sequenced by Sangon Biotech (Shanghai, China).

Expression of DEFB118 in E. coli

For expression, plasmids pET32a (+)-DEFB118 gene were transformed into E. coli Rosetta (DE3) cells using heat shock method. And then, Positive bacterial colonies were confirmed as the methods of construction of the expression vector. The selected positive bacterial were incubated, Once OD 600 reached 1.0, then 1.0 mM isopropyl β-d-1-thiogalactoside (IPTG) were added to induce protein expression. After incubation for 4 h at 28 °C, bacterial cells were harvested by centrifugation at 8000 × g for10 min at 4 °C and lysis by lysis buffer [500 mM NaCl, 20 mM Tris, 0.1% Triton X-100, 1 mM PMSF, Lysozyme 0.2 mg/mL, 10 U/mL DNase (pH 7.5)] for 30 min at 4 °C. Then, schizolytic cells were sonicated (4 s pulse and 8 s interval; 30 cycles; Sonics-Vibra cell, USA). And centrifuged at 15,000 × g for 30 min at 4 °C. The supernatant was collected and stored at -80 °Cuntil analysis.

Affinity Purification

The supernatant obtained above was filtered by 0.22 μm filter, and then applied to Ni 2+-IDA column (Sangon Biotech, China) and purified according specification. Briefly, added 10 resin volumes Binding Buffer (50 mM NaH_2PO_4,300 mM NaCl, pH 8.0) to wash way the impure protein, and then added 5 resin volumes Elution Buffer (50 mM NaH_2PO_4, 300 mM NaCl, 150 mM Imidazole, pH 8.0) to eluted the DEFB118 from the column. Then, protein concentration was quantified with the BCA assay (Beyotime, China). The purified DEFB118 was ran on 12%SDS-PAGE. The rest was stored at − 80 °C to analyze antimicrobial activities.

Antimicrobial Activity Assays

The minimal inhibitory concentration (MIC) of purified DEFB118 was measured by the microtiter broth dilution method [25]. E.coli DH5α, pathogenic E.coli K88+, S. typhimurium, S.aureus, B.subtilis were grown to 0.4 OD 600 nm at 37 °C in LB, Streptococcus was grown to 0.4 OD 600 nm at 37 °C in THY (Todd-Hewitt + yeast extract). The target cell culture was diluted to 1×10^5 CFUs/mL with same media respectively. A total of 100 μL of DEFB118 and 100 μL of cell suspension were added into each well. The activity of DEFB118 was tested over a concentration range of 512, 256, 128, 64, 32, 16, 8, 4, 2 and 1 mg/L, and all assays were tested in triplicated. Bacterial plates were incubated at 37 °C for 16 h, and the absorption of cell culture was recorded at 600 nm. MIC was defined as the lowest concentration of peptide at which there was no change in optical density.

Hemolytic Activity Assay

Hemolytic activity of DEFB118 was determined as described earlier [26]. In brief, erythrocytes from heparinized pig blood were washed thrice with cold PBS (pH 7.2) and resuspended to a concentration of 4% in saline. Erythrocytes were treated with different concentrations of DEFB118 (200 ∝l) in a 96-well plate and incubated at 37°C for 1 h. The plate was centrifuged at 1000 rpm for 5 min, and supernatants were transferred to a fresh plate. Absorbance at 414 nm of saline and 0.1% TritonX-100-treated erythrocytes served as 0 and 100% hemolysis controls, respectively.

Cell culture

Intestinal porcine epithelial cells (IPEC-J2) were cultured in 75 cm^2 cell culture flask in DMEM-F12 with 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin. 1×10^5 cells/well were seeded in 12-well plates and grown to ~ 60% confluence at 37 °C in a CO$_2$ incubator (5% v/v), then incubated with antimicrobial peptides DEFB118 (25 μg/mL) for 12 h (DEFB118, DEFB118 + E.coli k88). Cells were challenged with 1×10^6 CFU/well E. coli K88 for 1 h (DEFB118 + E.coli k88, E.coli k88), control cells were cultured in a culture medium of 2% serum (without any antibiotics) without any treatment. Total cellular RNA was collected using RNAiso Plus (Takara, Dalian, China).

Cytotoxicity assay

The cytotoxicity of DEFB118 was measured according to previously a study [27]. Briefly, IPEC-J2 cells were cultured in DMEM-F12 with 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin for 48 h and then resuspended to 10^5 cells/mL in FBS free DMEM-F12 media. A volume of 100 μL of cells was aliquoted into sterile flat-bottomed 96-well plates (Corning, USA). The DEFB118 (25 μg/mL) was added to the cells and incubated at 37 °C/5% CO$_2$ for 24 h (DEFB118, DEFB118 + E.coli k88). Then cells were challenged with 1×10^6 CFU/well E. coli K88 for 1 h (DEFB118 + E.coli k88, E.coli k88), control cells were cultured in complete medium without any treatment. Cell viability was evaluated with the CCK-8 assay (Beyotime, Shanghai, China) according to the manufacturer's instructions.

Assessment of apoptosis by flow cytometry

In order to evaluate the protective effect of DEFB118 against E.coli k88, IPEC-J2 cells were grown to ~ 60% confluence at 37 °C in a CO$_2$ incubator (5% v/v). Then incubated with DEFB118 (25 μg/mL) for 12 h (DEFB118, DEFB118 + E.coli k88). Cells were challenged with 1×10^6 CFU/well E. coli K88 for 2.5 h (DEFB118 + E.coli k88, E.coli k88), control cells were cultured in a culture medium of 2% serum (without any antibiotics) without any treatment. Treated cells were harvested and labeled with an anti-Annexin V-FITC Apoptosis Detection Kit (BD Biosciences, USA). Floating cells were collected, then attached cells were washed with 0.01 M PBS and trypsinized for 2 min. Finally, trypsinized cells and floating cells were added together to centrifuge at 350 g for 10 min, then stained with AnnexinV-FITC and propidium iodide (PI). The intensity of the markers was examined by flow cytometry (FACSC antoII, BD Biosciences, USA).All flow cytometric data were analyzed by using FlowJo software (BD Biosciences, USA).

RNA extraction and RT-PCR

IPEC-J2 cells were harvested and the total RNA was extracted using RNAiso Plus (Takara, Dalian, China) according to manufacturer's instructions. The quantity and quality of the isolated RNA were determined by absorbance at 260 and 280 nm [28]. And then cDNA was synthesized using a Reverse Transcriptase kit (Takara, Dalian, China). Briefly, quantitative PCR was performed by QuanStudio 6 Flex Real-Time PCR detection system (Applied Biosystems, Foster City, CA, USA) with a total of 10 μL of assay solution containing 5 μL SYBR Green mix (Takara), 0.2 μL Rox, 3 μL deionized H$_2$O, 1 μL cDNA template, and 0.4 μL each of forward and reverse primers (Qingke, China). The relative gene expressions compared with the housekeeping gene β-actin were calculated by 2^{-CT} [29].

Statistics analysis

All statistical analysis was performed using SPSS 21.0 software. Data were expressed as the mean ± standard error of the mean (SEM). Statistical analysis of treatment of IPEC-J2 and cytotoxicity were carried out using two-way ANOVA followed by Duncan's multiple comparisons test. Image production using GraphPad Prism software (Version 7. GraphPad Software Inc., CA, USA)..

Results

Comparison of the DEFB118 nucleotide sequences

Blast analysis of the synthesized DEFB118 sequence was performed by using the DNAMAN 8.0. Results showed that the synthesized DEFB118 sequence was consistent with the published sequence (Fig. S1). Both contain a 372-bp open reading frame, which encodes a 123-amino acid DEFB118 mature protein. Structural analysis by using the "SWISS model" showed that the DEFB118 protein exhibited a classic beta-ring conformation (Fig. 1A). Amino acid sequence analysis showed that the amino acid sequence of DEFB118 is highly conserved (Fig. 1B). The human DEFB118 sequence is more than 97% identical to the sequences obtained from Pan troglodytes, Gorilla gorilla, and Nomascus leucogenys. Phylogenetic tree analysis showed that the human DEFB118 is close to Gorilla gorilla (Fig. 1C).

Expression and purification of the recombinant DEFB118

The DEFB118 gene with two designated restriction enzyme sites (EcoRI/NotI) was artificially synthesized. The two restriction enzyme sites allow directional cloning of the DEFB118 gene into the pET32a expression vector. A 312-bp fragment was observed after double digestion of recombinant plasmid with the two restriction enzymes (Fig.S2A). The recombinant plasmids were transformed into the E. coli Rosetta (DE3) and the positive clones were selected by PCR (Fig.S2B). The most desired strain was chosen for small-scale induction by using 1 mmol/L IPTG at 28 °C. We found that the induction time significantly affected the expression level. As shown in Fig. 2A, the E. coli achieved a maximal yield of the DEFB118 protein after 4 h induction (more than 250 μg/mL). The molecular weight of recombinant DEFB118 was estimated by SDS-PAGE to be 30 kDa (Fig. 2A and B). The crude protein collected from ultrasonically-disrupted bacteria was purified by using Ni^{2+}-IDA affinity chromatography (Fig. 2B). The target band was collected and amino acid sequence of DEFB118 was identified by using the mass spectrometry (MALDI-TOF/TOF). As shown in Fig. 3, the protein sequence of the recombinant DEFB118 protein 100% matches the sequence of human DEFB118 (NP_473453.1).

Antibacterial activity of the DEFB118

The antibacterial activities of DEFB118 was investigated by using Gram-negative and Gram-positive bacteria strains. As shown in Table 1, DEFB118 showed strong antibacterial activity against Gram-negative bacteria such as the E. coli K88 and E. coli DH5α with a MIC of 4 mg/L. DEFB118 also showed

antibacterial activity against the S. typhimurium (with a MIC of 8 mg/L). Moreover, the DEFB118 showed strong antibacterial activities against Gram-positive bacteria such as the S. aureus and B. subtilis with a MIC of 4 μg/mL.

Table.1 MIC of DEFB118 produced by E. coli Rosetta (DE3).

Strain	MIC (μg/mL)
Gram-negative bacteria	
E. coli DH5α 32	4
Pathogenic E. coli K88	4
Salmonella typhimurium CICC14028	8
Gram-positive bacteria	
Streptococcus	32
Staphylococcus aureus CICC23656	4
Bacillus subtilis	4

MIC minimal inhibitory concentration.

Hemolytic activity of the DEFB118

Erythrocytes were collected from fresh porcine blood and incubated with different concentrations (0–256 mg/L) of DEFB118 for 1 h. As compared to the TritonX-100, the DEFB118 showed no significant hemolytic activity at all concentrations (Fig. 4).

Influences of DEFB118 on cell viability, apoptosis, and inflammatory response in IPEC-J2 cells

As shown in Fig. 5, E. coli K88 challenge decreased the viability of the IPEC-J2 cells. However, DEFB118 treatment significantly elevated the cell viability ($P < 0.05$). As compared to the control group, E. coli K88 challenge significantly elevated the apoptosis rate in the IPEC-J2 cells (Fig. 6). However, DEFB118 treatment significantly decreased late apoptosis rate in the E. coli K88-challenged cells ($P < 0.05$). Interestingly, E. coli K88 challenge significantly elevated the expression levels of inflammatory cytokines such as the IL-1β, IL-6, and TNF-α in the IPEC-J2 cells (Fig. 7A). However, DEFB118 treatment down-regulated their expression levels in the E. coli K88-challenged cells ($P < 0.05$). Moreover, DEFB118 treatment significantly down-regulated the expression level of caspase3 in the E. coli K88-challenged cells ($P < 0.05$).

Discussion

In last decades, the uses and misuse of antibiotics has led to the developing of antibiotic resistance (AMR), which is one of the biggest threats to global public health [30]. The World Health Organization (WHO) predicts that there will be 10 million deaths due to AMR in 2050 [31]. Therefore, substitute for conventionally used antibiotics has attracted considerable research interest worldwide. Defensins are a family of host defense peptides present in vertebrates, invertebrates and plants. Currently, the β-defensins has attracted considerable research interest since it has been reported to show a broad-spectrum antimicrobial activity and participate in the regulation of immune functions [32–34]. DEFB118 is a newly

identified human beta-defensin, which is highly expressed in the epithelial cells of different ducts and most abundant in the caput epithelium [23]. However, the exact role of DEFB118 is poorly understood. Moreover, direct isolation of the DEFB118 from human tissues is not commercially feasible because of its low quantity [35]. In the present study, the DEFB118 was obtained by using heterologous expression, and the recombinant DEFB118 was purified and fully characterized.

The recombinant DEFB118 was estimated by SDS-PAGE to be 30 kDa, and MALDI-TOF analysis indicated that its amino acid sequence is consistent with human beta-defensin 118. It is a well-known fact that the culture conditions such as the induction times and temperatures will affect the yield of protein expression for heterologous expression systems [36]. In this study, the highest yield of DEFB118 in E. coli was observed after 4 h IPTG induction. This is different from a previous study which achieved a maximal expression after 2 h induction [36].The difference may result from the use of different bacteria strains [37]. Interestingly, antimicrobial activity assays showed that DEFB118 has significant antimicrobial activity against both the Gram-positive bacteria (S. aureus and B. subtilis) and Gram-negative bacteria (E. coli K88 and E. coli DH5α).The MIC for DEFB118 against S. aureus and E. coli K88 was 4 mg/L, which is lower than beta defenses obtained in previous study [38]. These results are also consistent with a previous report on the DEFB118, and both results indicated that the DEFB118 had a broad-spectrum of antibacterial activities [37]. Importantly, hemolytic assays showed that DEFB118 had no detrimental impact on cell viability, indicating that it is safe for human use and may be tentatively used as a substitute for conventionally used antibiotics.

Enteropathogens Escherichia coli (ETEC) adheres to the intestinal epithelium and induces severe diarrhea and intestinal inflammation [39]. In addition to their antibacterial activities, evidence is accumulating to show that the β-defensins can also function as an immunomodulator for mammalian animals. For instance, human β-defensin 1, β-defensin 2 and β-defensin 3 were found to have both the anti-inflammatory and immunoregulatory functions [40–43]. In the present study, we explored the influence of DEFB118 on inflammatory responses in the intestinal epithelial cells exposure to ETEC K88 [44]. We found that E. coliK88 challenge significantly decreased the cell viability and elevated the apoptosis rate in the PIEC-J2 cells. This is consistent with previous studies that microbial infections or stresses increases the apoptosis of the intestinal epithelial cells [45]. Interestingly, DEFB118 significantly decreased the apoptosis in the ETEC K88-challenged cells. This is probably due to the down-regulation of several critical inflammatory cytokines such as the IL-1β and TNF-α. Previous study indicated overproduction of inflammatory cytokines has resulted in changes of whole-body metabolism and disruption of the tissues such as the muscle and intestinal mucosa [46, 47]. Moreover, both the IL-1β and TNF-α can induce cell apoptosis via intrinsic mitochondrial apoptotic pathway [48, 49]. In the present study, ETEC K88 challenge significantly elevated their expression levels in the PIEC-J2 cells. However, DFEB118 treatment resulted in significant down-regulation of the two critical inflammatory cytokines. The result is also consistent with previous studies on different animal species [14, 50].

Caspases are proteolytic enzymes that mediate programmed cell death (apoptosis) and are highly conserved among different species [51]. The family of caspases can be further divided into initiator

(caspases 8, 9, 10) and executioner (caspases 3, 6, 7) [52]. Among these executioners, caspase3 is extremely important as both intrinsic and extrinsic pathways converge at caspase3 [53].In this study, E. coli k88 challenge significantly elevated the expression levels of caspase3 in the PIEC-J2 cells, which was consistent with previous study on piglets [54]. However, DEFB118 can down-regulate the expression levels of caspase3. However, DEFB118 significantly down-regulated the expression level of caspase3. This is probably due to the down-regulation of inflammatory cytokines (i.e. TNF-α) since they were reported to induce apoptosis via activation of the caspase system [48, 49].

In conclusion, the DEFB118 shows broad-spectrum of antimicrobial activities and few hemolytic activity and cytotoxicity. Additionally, DEFB118 increases the cell viability in the intestinal epithelial cells exposure to E. coli K88, which was associated with decreased cell apoptosis and down-regulation of inflammatory cytokines. The anti-bacterial and anti-inflammatory properties of DEFB118 may allow it a potential substitute for conventionally used antibiotics or drugs.

Abbreviations

AMPs: Antimicrobial peptides; AST: Glutinous straw transaminase; DEFB118: β-defensin 118; HDP: host defence peptide; *E. coli: Escherichia coli*; *E. coli* DE3: *Escherichia coli* Rosetta; *S. typhimurium: Salmonella typhimurium* TCC14028 ; *S. aureus: Staphylococcus aureus* CICC23656; *B. subtilis: Bacillus subtilis*; IPTG: Isopropyl β-d-1-thiogalactoside; MIC: minimal inhibitory concentration; Caspase3: Cysteinyl aspartate specific proteinase 3; Caspase8: Cysteinyl aspartate specific proteinase 8; Caspase9: Cysteinyl aspartate specific proteinase 9; ETEC: Enterotoxigenic Escherichia coli; IL-1β: Interleukin 1 beta; IL-6: Interleukin 6; TNF-α: Tumor necrosis factor alpha; ZO-1: Zonula occludens-1;

Declarations

Authors' contributions

QL and KHX conceived the study, performed the experiment, performed data analysis, and contributed to drafting the manuscript. QL carried out the animal experiment. DWC, BY, XBM, JY, PZ, JQL, YHL, HY and JY conceived the experiment and proofread the manuscript. All authors read and approved the final manuscript.

animal Feed Biotechnology of Sichuan Province (2016TD0028).

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

All experimental procedures and animal care were carried out in compliance with the regulations of the Animal Care Committee of Sichuan Agricultural University (No. 20180701).

Consent for publication

Not applicable.

Conflicts of interest

The authors declare that they have no competing interests.

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Additional Files

Fig. S1. Strategy of cloning of DEFB118 gene.

The CDS sequence without signal peptide of PNKL was cloned, *EcoR* I and *Not* I was insert in 5' and 3' respectively.

Fig. S2. Results from agarose gel electrophoresis (EcoRI/Not I digestion map).

a Digestion of the recombinant expression vectors. Lane M_1 DNA marker (DL 10 000), Lanes 1 DEFB118 (without digestion). Lanes 2 DEFB118 (digestion with EcoRI and NotI) Lane M_2 DNA marker (DL 2000). b RT-PCR of DEFB118. Lane M DNA marker(DL 2000), Lanes 1-4 DEFB118 (production of five tubes of reaction solution).
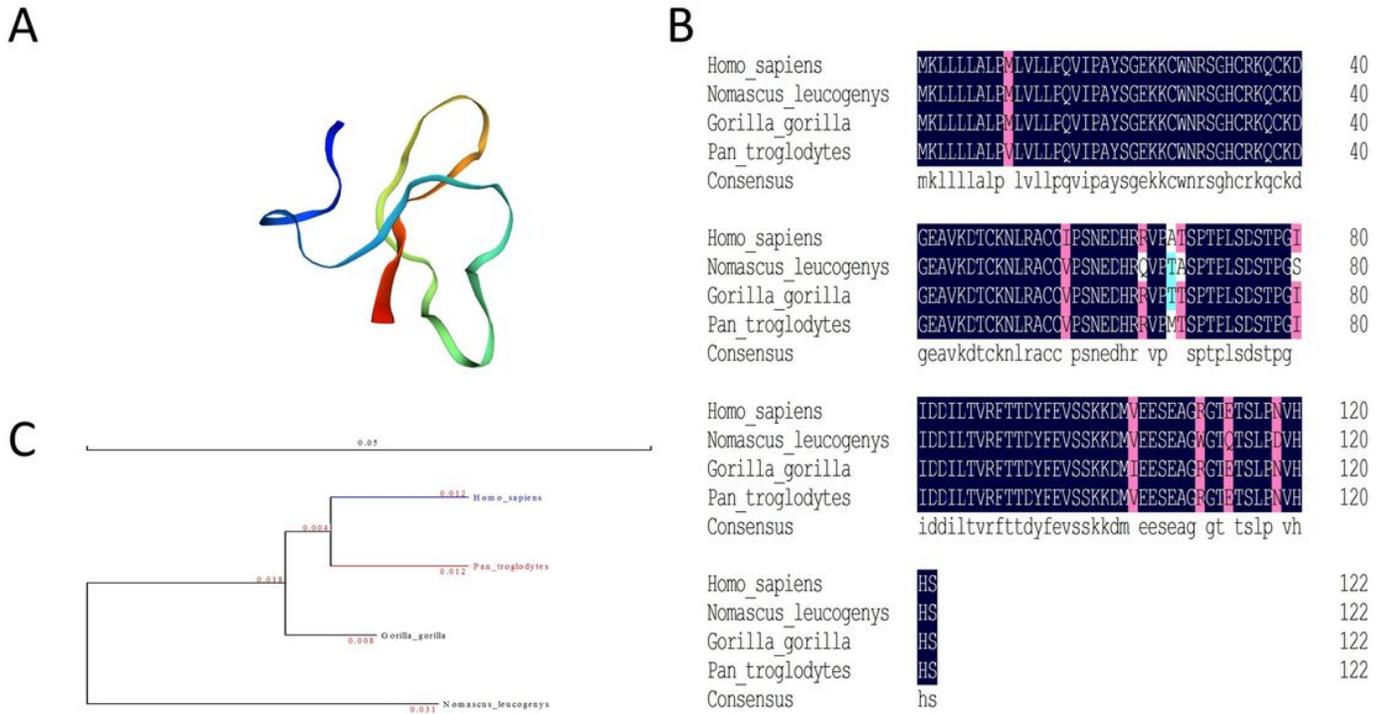
Figures

Figure 1

Phylogenetic analysis of beta defensin 118. a, prediction model of DEFB118; b, phylogenetic analysis of beta defensin 118 in Homo sapiens, Pan troglodytes, Gorilla gorilla and Nomascus leucogenys were performed by DNAMAN 8.0; c, amino acid sequences of beta defensin 118 in Homo sapiens, Pan troglodytes, Gorilla gorilla and Nomascus leucogenys were aligned by DNAMAN 8.0.
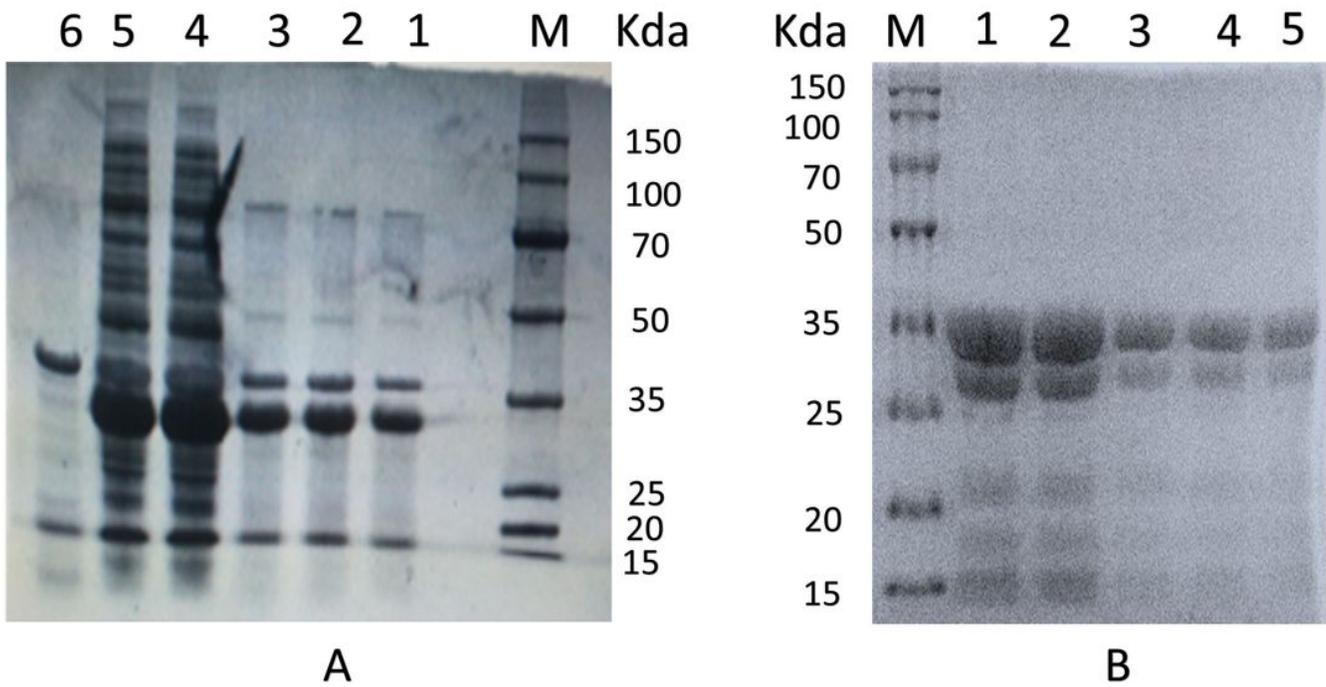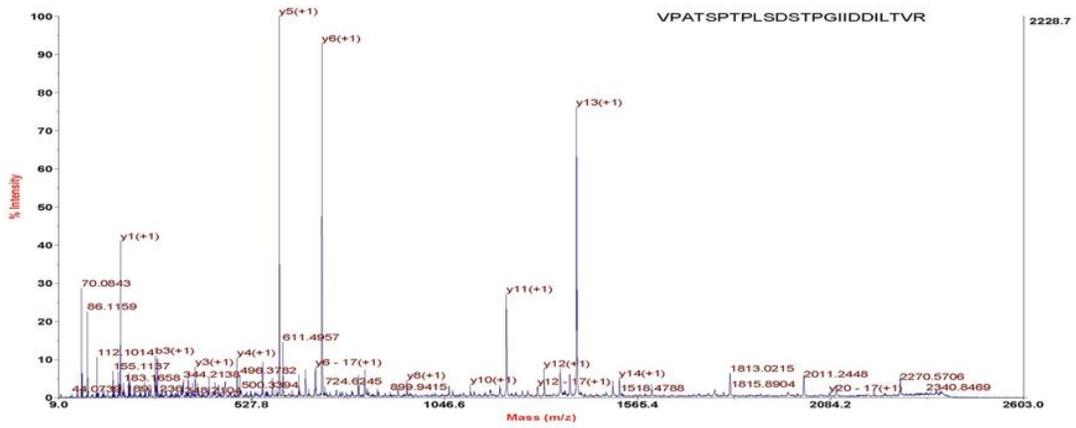
Figure 2

SDS-PAGE analysis of DEFB118 produced by E. coli Rosetta. a SDS-PAGE of DEFB118 from E. coli Rosetta. M protein markers (DL 150 Kda), Lane 1, E. coli Origami B (DE3)-pET32a (+) induced by 1 mmol/L IPTG for 10 h at 28 °C, Lane 2, E. coli Origami B (DE3)-pET32a (+) induced by 1 mmol/L IPTG for 8 h at 28 °C, Lane 3, E. coli Origami B (DE3)-pET32a (+) induced by 1 mmol/L IPTG for 6 h at 28 °C, Lane 4, E. coli Origami B (DE3)-pET32a (+) induced by 1 mmol/L IPTG for 4 h at 28 °C, Lane 5, E. coli Origami B (DE3)-pET32a (+) induced by 1 mmol/L IPTG for 2 h at 28 °C, Lane 6, E. coli Origami B (DE3)-pET32a (+) (non-induced); b Purification of DEFB118. M protein markers (DL 150 Kda), Lane 1-2 DEFB118 (1.2 mg/mL) purified by Ni 2+ -IDA affinity chromatography, Lane 3-5 DEFB118 (0.6 mg/mL) purified by Ni 2+ -IDA affinity chromatography.

Figure 3

Mass spectrometry identification of DEFB118. a, peak figure of amino acid fragments; b, through searching uniprot-Homo-sapiens, DEFB118 sequence had a math with NP_473453.1 (show in red)
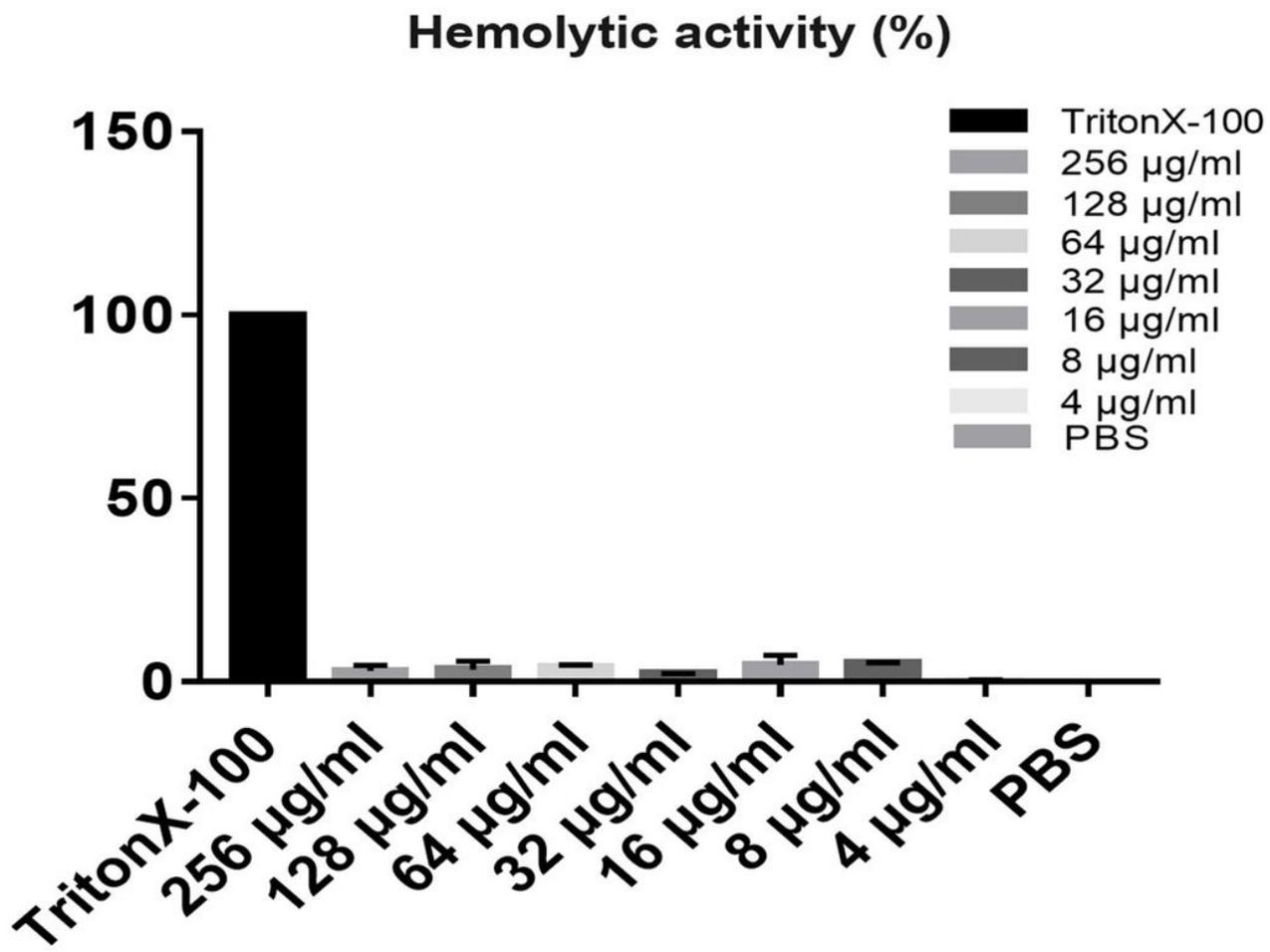
Hemolytic activity (%)

Legend:
- TritonX-100
- 256 µg/ml
- 128 µg/ml
- 64 µg/ml
- 32 µg/ml
- 16 µg/ml
- 8 µg/ml
- 4 µg/ml
- PBS

X-axis categories: TritonX-100, 256 µg/ml, 128 µg/ml, 64 µg/ml, 32 µg/ml, 16 µg/ml, 8 µg/ml, 4 µg/ml, PBS

Figure 4

Hemolytic activity of recombinant of DEFB118. DEFB118 256 µg/mL DEFB118, Triton X-100 1% Triton X-100, PBS 10 Mm PBS (pH 7.3).

Figure 5
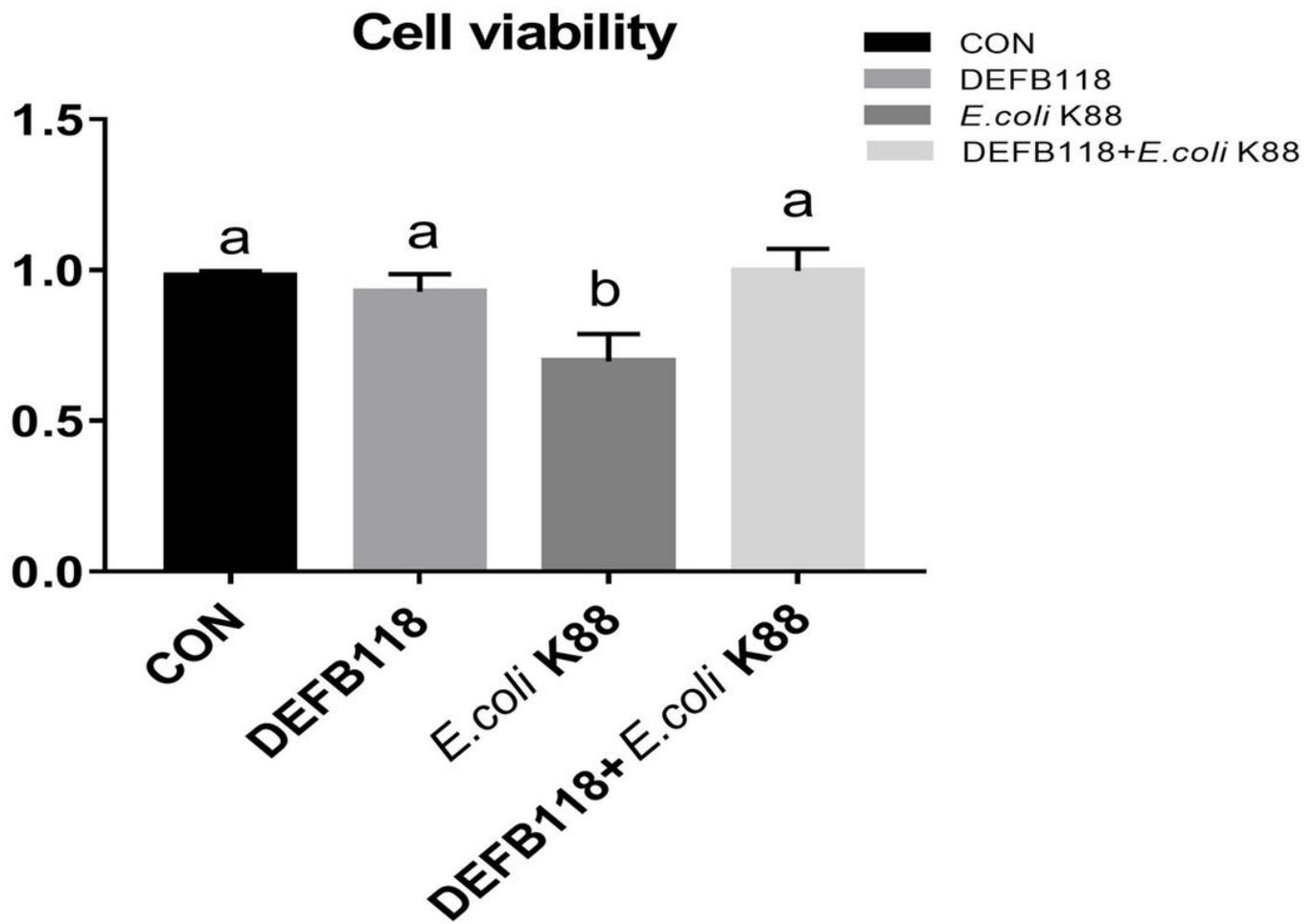
Influence of DEFB118 on E. coli K88-induced cell viability in IPEC-J2 cells. Viability of IPEC-J2 cells was determined by incubation with CCK8 for 1 h after different treatments. a-b Values within a column differ if they do not share a common superscript ($P < 0.05$).
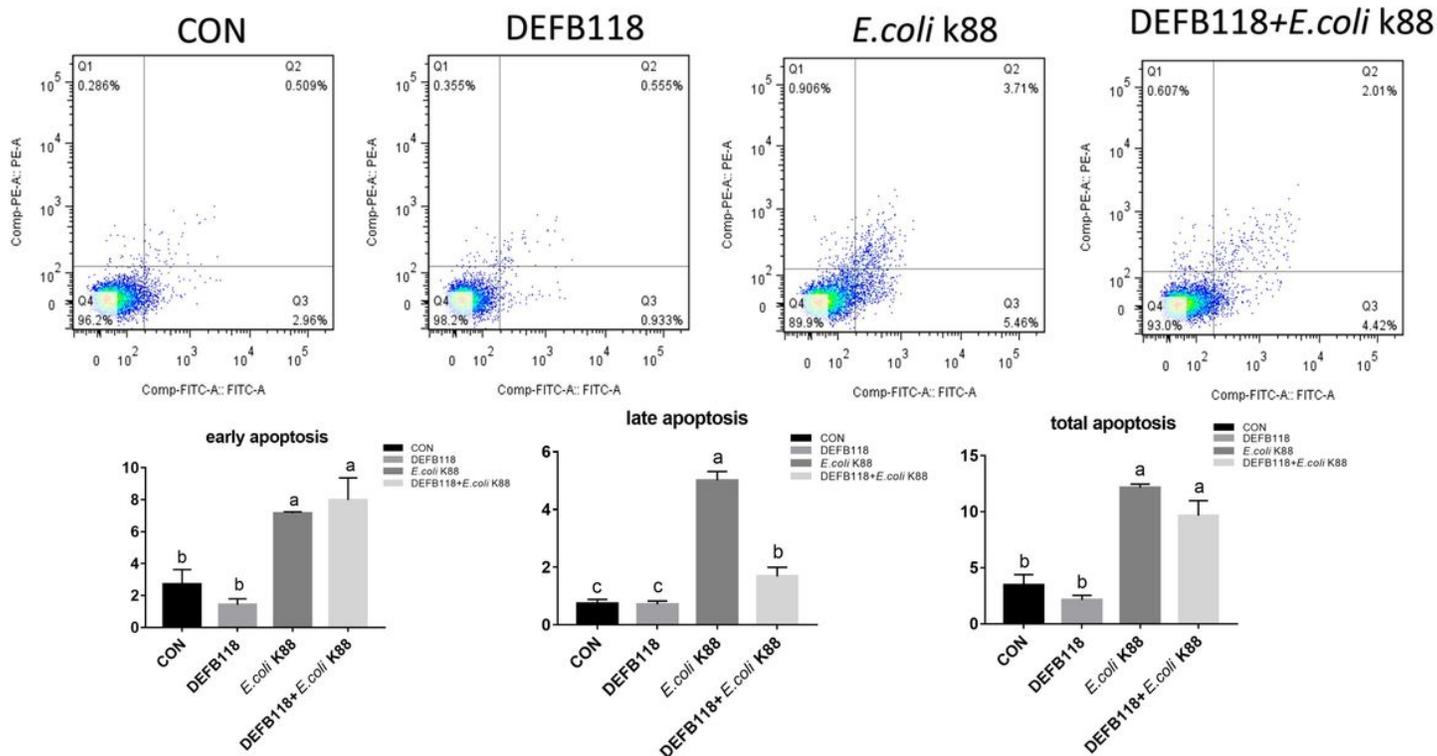
Figure 6

Influence of DEFB118 on E. coli K88-induced apoptosis in IPEC-J2 cells. Cell distribution analysis of apoptosis of IPEC-J2 cells treated with DEFB118, E. coli K88, and DEFB118 plus E. coli K88. In each diagram, Q1 represents the percentage of non-viable, necrotic cells, Q2 represents the percentage of late apoptotic IPEC-J2 cells, Q3 represents the percentages of early apoptotic IPEC-J2 cells and Q4 represents the percentage of live IPEC-J2 cells. The statistical analysis of cell distribution data among samples, total apoptotic cells included Q2 with Q3. a-c Values within a column differ if they do not share a common superscript (P < 0.05).
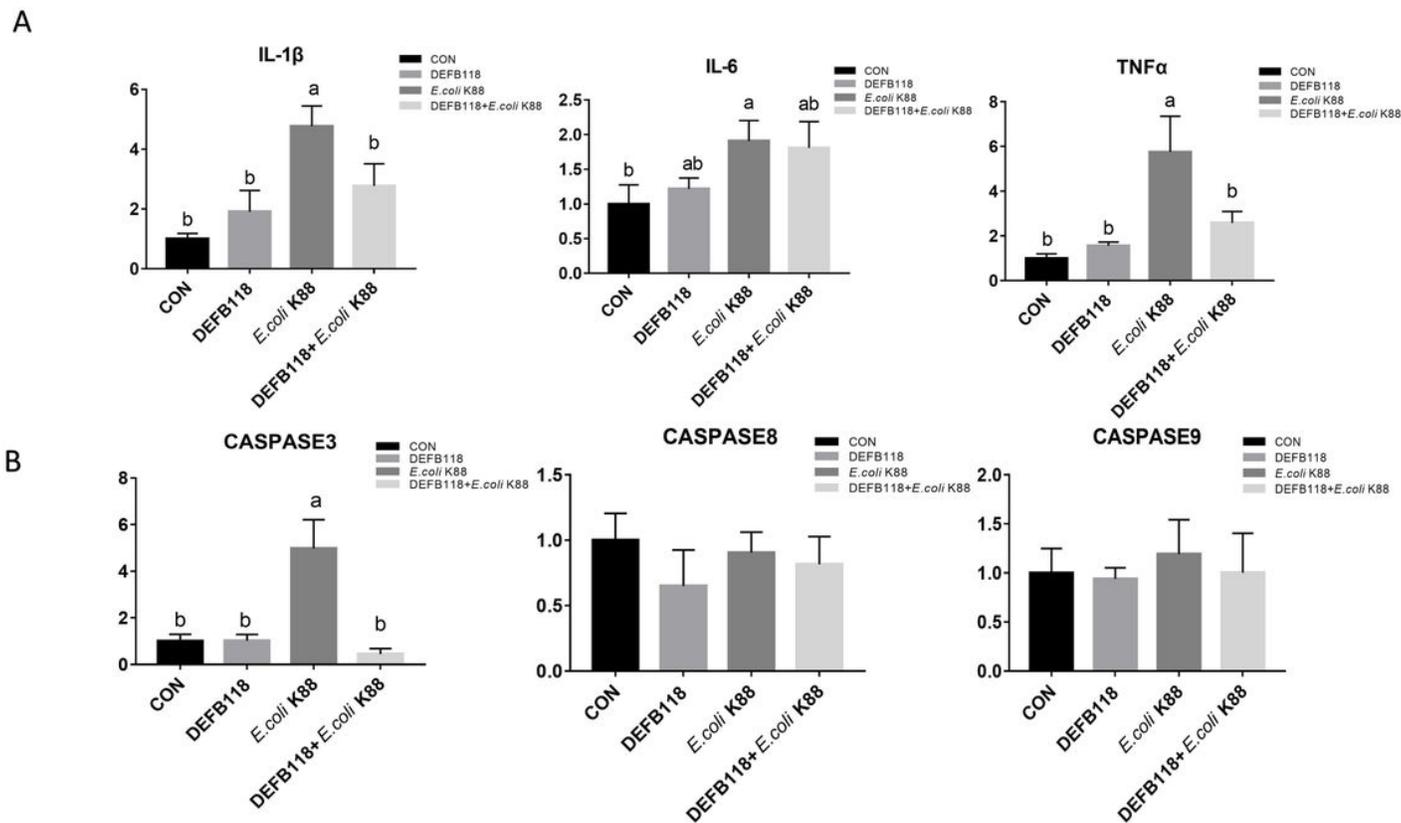
Figure 7

Influence of DEFB118 on E. coli K88-induced inflammatory responses in IPEC-J2 cells. Total RNA was extracted from IPEC-J2 cells and the expression of related genes were measured by real-time fluorescence PCR. The target gene mRNA expression level was calculated using the 2−ΔΔCt method. a, proinflammatory cytokine. b, Apoptotic factor. a-b Values within a column differ if they do not share a common superscript (P< 0.05). IL-1β, interleukin 1 beta; IL-6, interleukin 6; TNF-, tumor necrosis factor alpha.

Supplementary Files

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- Fig.S1.jpg
- Fig.S2.jpg