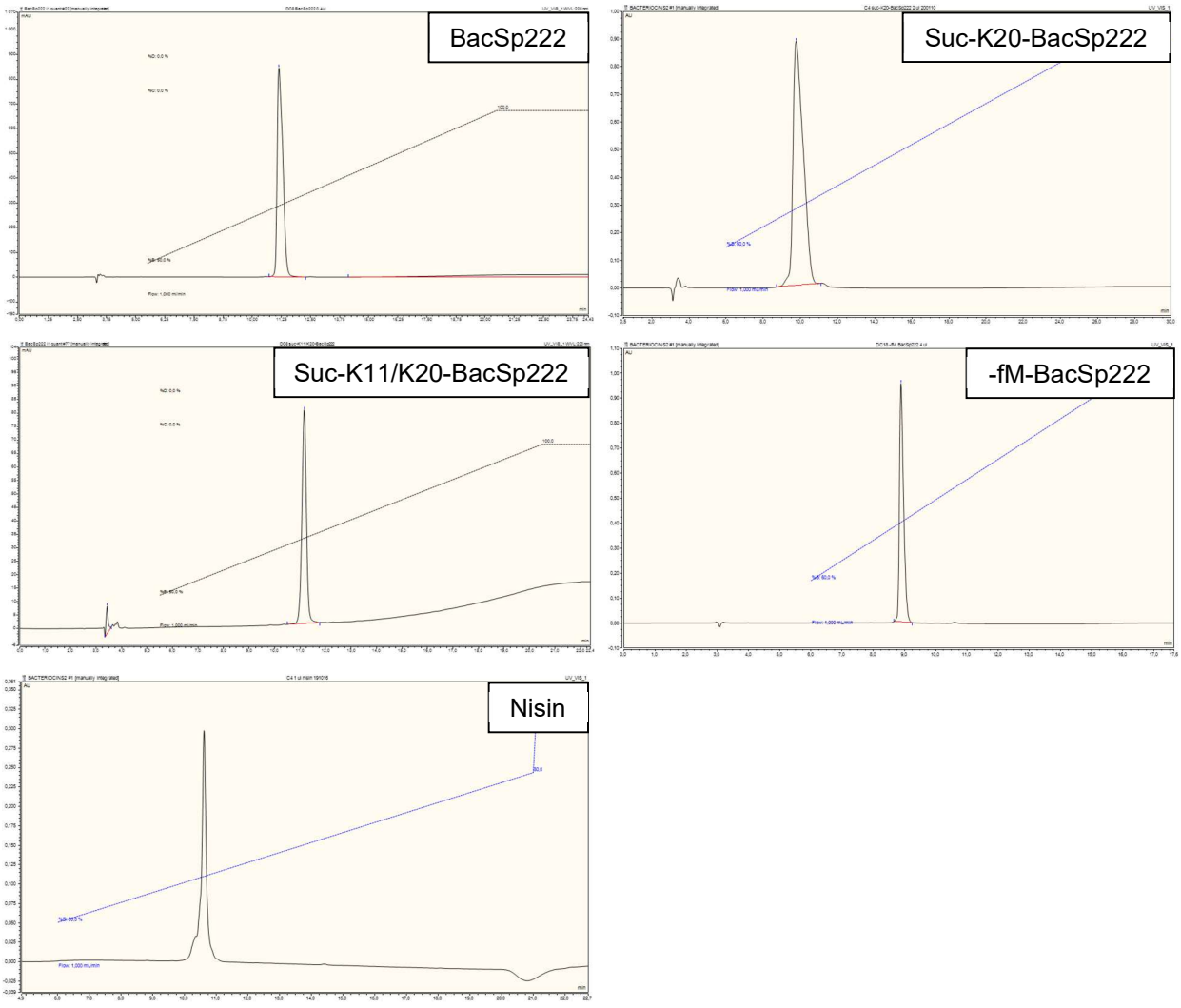
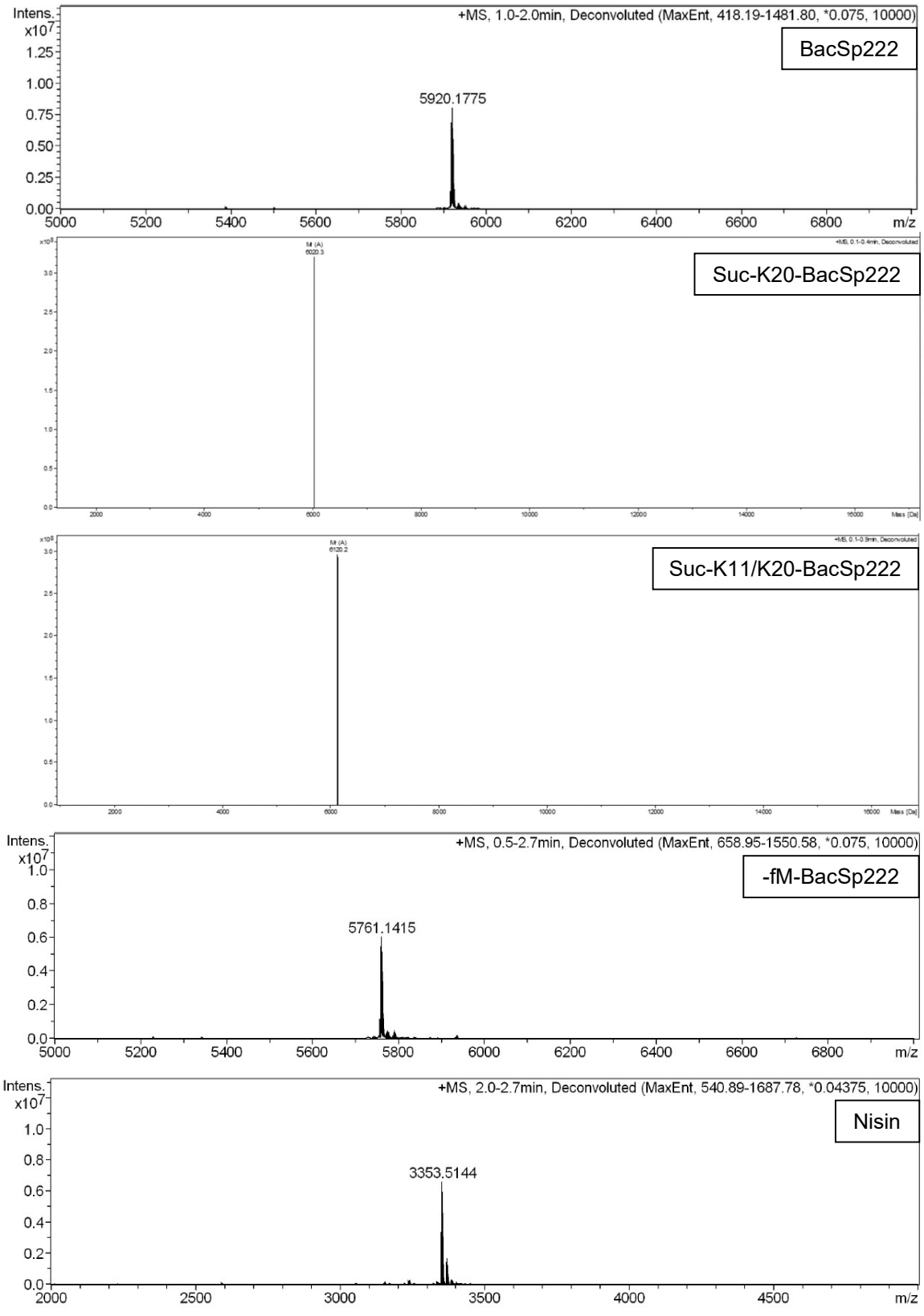
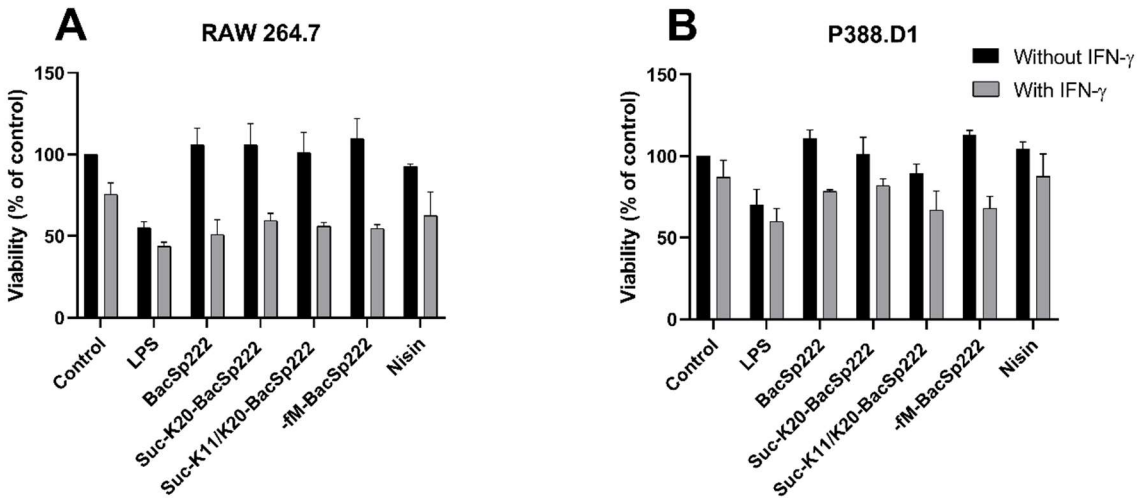


Supplementary Figure 1 The results of analytical RP-HPLC separations of peptide bacteriocins (BacSp222, suc-K20-BacSp222, suc-K11/K20-BacSp222, -fM-BacSp222 and nisin) used in the study. The peptides were separated using different C4, C8 or C18 reversed-phase columns under conditions described in section Materials and Methods - Peptides and Protein Chemistry Techniques.

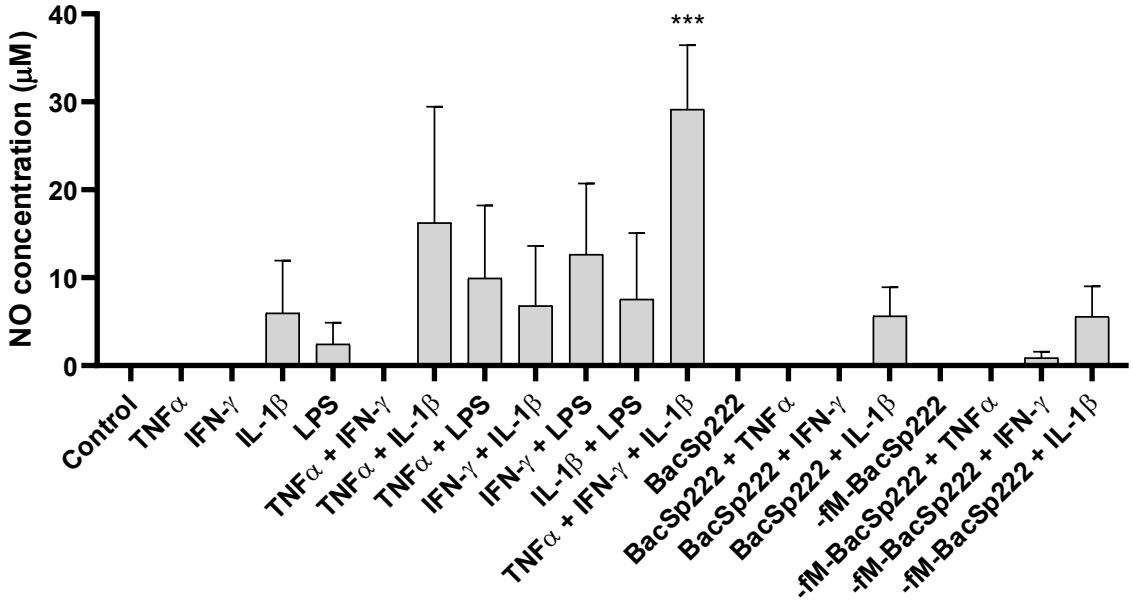


Supplementary Figure 2 The results of mass spectrometry analyses of peptide bacteriocins (BacSp222, suc-K20-BacSp222, suc-K11/K20-BacSp222, -fM-BacSp222 and nisin) used in the study. Presented mass spectra were measured using a HTC Ultra ETD II or micrOTOF-Q mass spectrometers (Bruker, Germany).



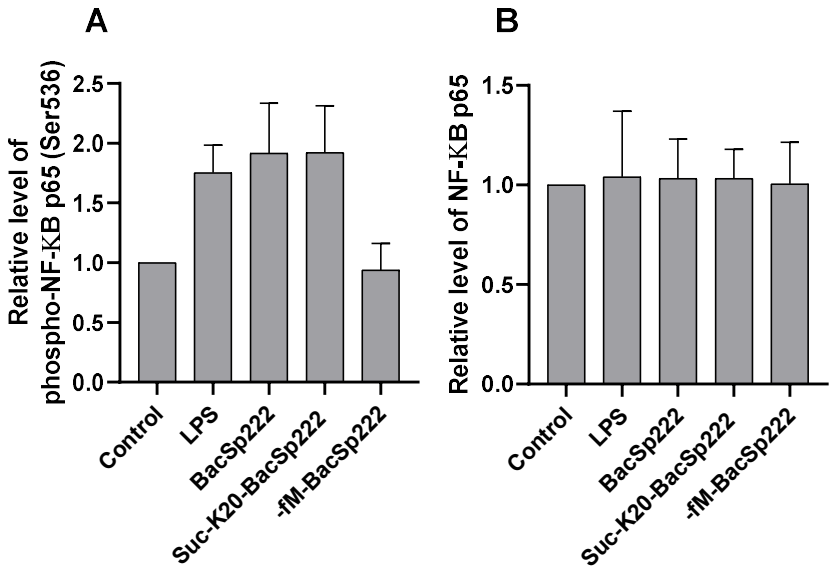
Supplementary Figure 3 The viability of RAW 264.7 (panel A) and P388.D1 (panel B) cells stimulated with various forms of bacteriocin. The cells were incubated in medium (control) or medium supplemented with LPS, BacSp222, suc-K20- BacSp222, suc-K11/K20- BacSp222, -fM-BacSp222 or nisin for 24 h. Then, the viability was measured with an MTT assay. The bars represent the mean ± SEM (n=3).

Abbreviations: LPS – lipopolysaccharide.



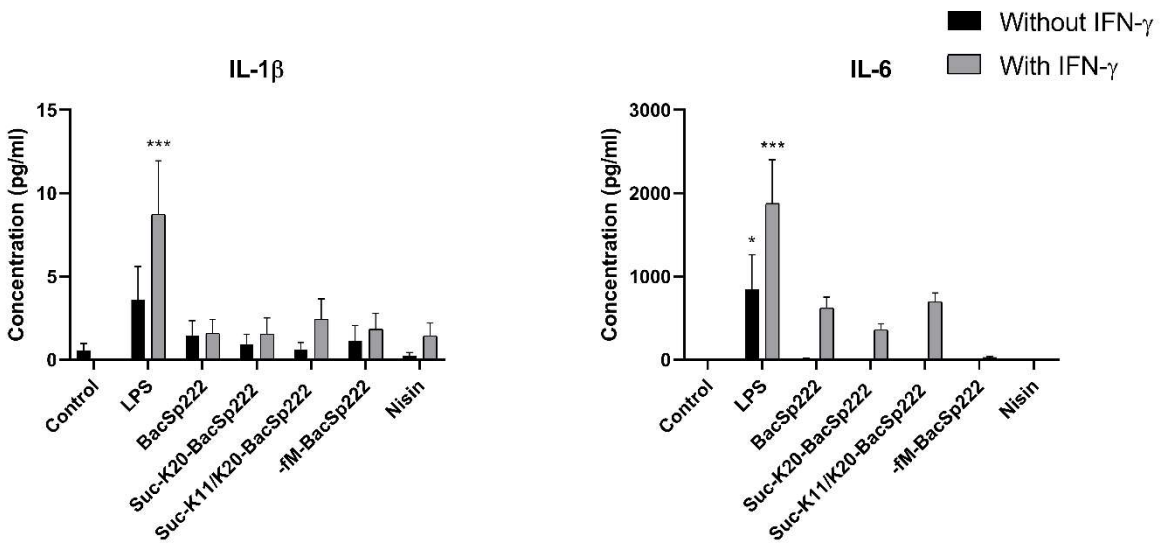
Supplementary Figure 4 Bacteriocins do not potentiate NO production by MBE cells. Determination of NO concentration measured by Griess reaction in cultured media collected from the stimulated cells. The bars represent the mean ± SEM (n=3). *** $p<0.001$ vs control.

Abbreviations: NO – nitrite oxide; iNOS – inducible NO synthase.



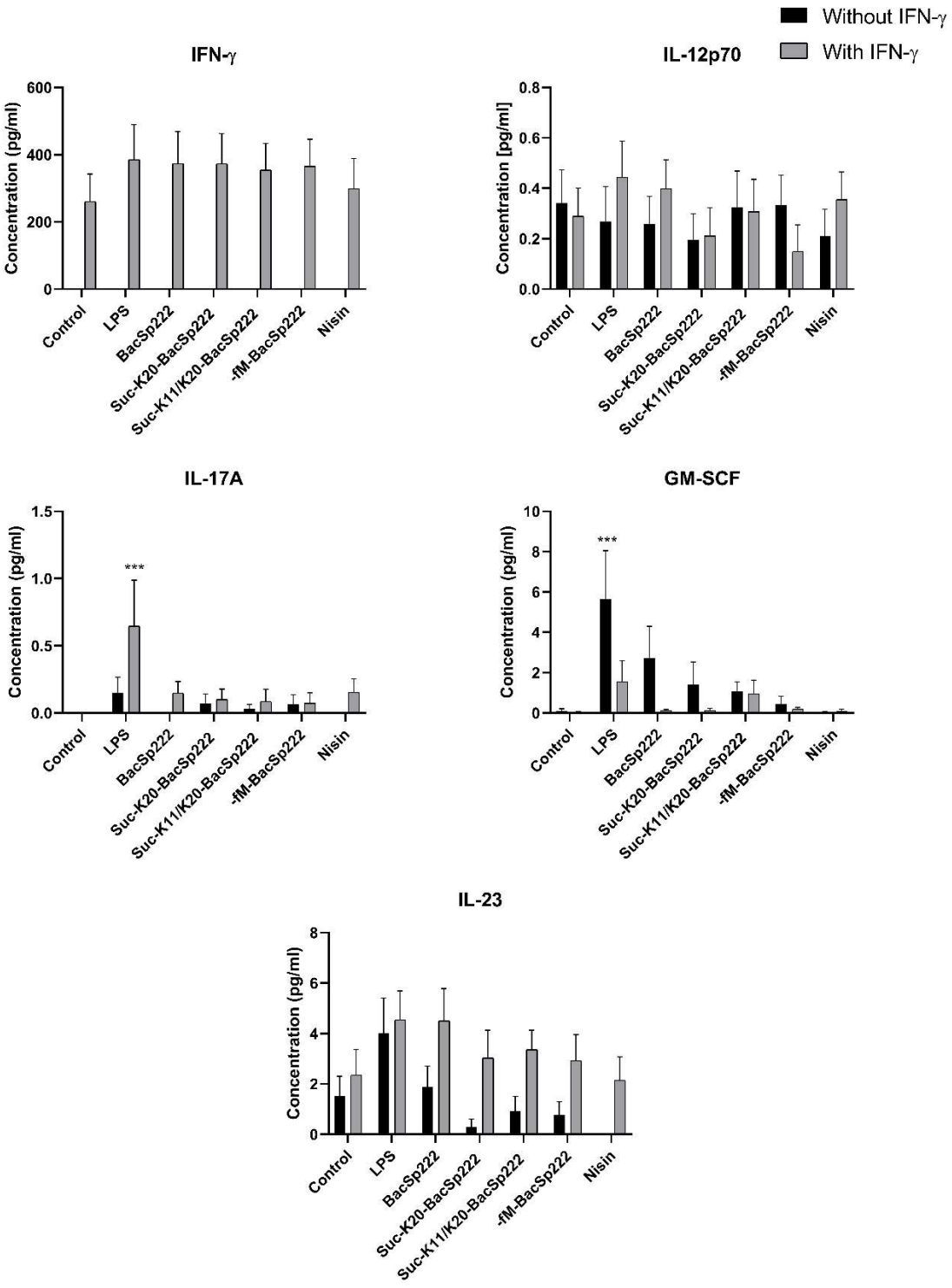
Supplementary Figure 5 Densitometric analysis of WB results demonstrating activation of NF-κB in P388.D1 cells exposed to different forms of BacSp222. The cells were incubated for 30 minutes in the control medium and in the medium supplemented with LPS, BacSp222, suc-K20- BacSp222, or with -fM-BacSp222. The cells were then lysed, and the lysates were analyzed using the WB method. The intensity of the bands corresponding to NF-κB was quantitated by the densitometric analysis (ImageJ software v. 1.53c, National Institute of Health). A: Intracellular level of the phosphorylated p65 subunit detected using an antibody specific to phosphorylated p65 Ser536. B: Intracellular level of the p65 subunit in the cells. The bars represent the mean ± SEM (n=3).

Abbreviations: LPS – lipopolysaccharide, WB – western blotting.



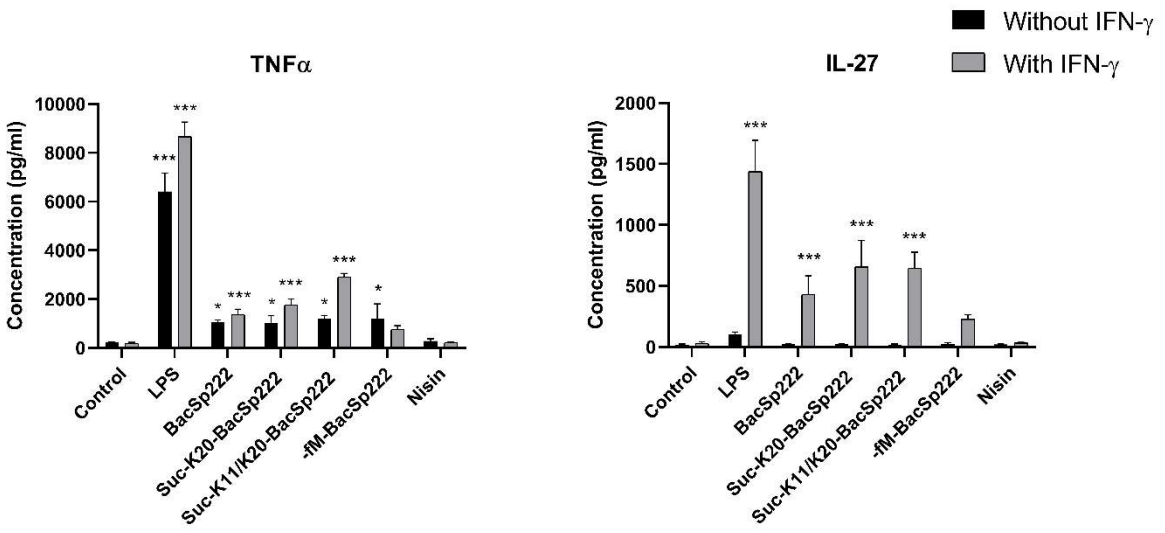
Supplementary Figure 6 The production of IL-1β and IL-6 by RAW 264.7 cells exposed to various forms of bacteriocin BacSp222. The cells were incubated in medium (control), medium supplemented with LPS, BacSp222, suc-K20- BacSp222, suc-K11/K20- BacSp222, -fM-BacSp222 or nisin for 24 h. After 24h, the media were collected and subjected to flow cytometry analysis using LEGEND/Plex Mouse Inflammation Panel kit to determine cytokines concentrations. The bars represent the mean ± SEM (n=8). *** $p<0.001$ vs control, * $p<0.05$ vs control.

Abbreviations: LPS – lipopolysaccharide.



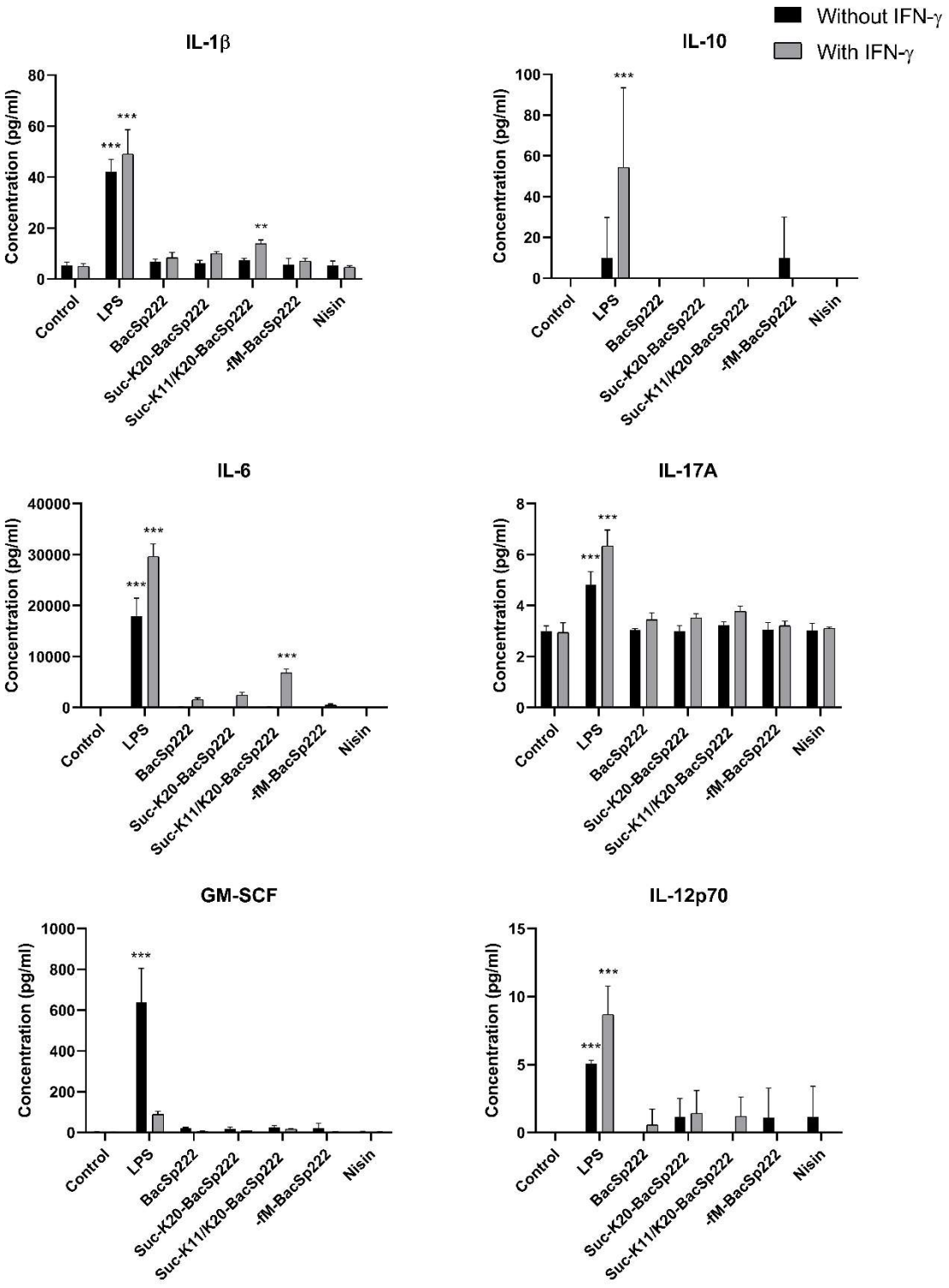
Supplementary Figure 7 The production of selected cytokines by RAW 264.7 cells exposed to various forms of bacteriocin BacSp222. The cells were incubated in medium (control), medium supplemented with LPS, BacSp222, suc-K20-BacSp222, suc-K11/K20-BacSp222, -fM-BacSp222, or nisin. After 24h the media were collected and subjected to flow cytometry analyses using LEGEND/Plex Mouse Inflammation Panel kit to determine cytokine concentrations. The bars represent the mean ± SEM (n=8). *** $p<0.001$ vs control.

Abbreviations: LPS – lipopolysaccharide.



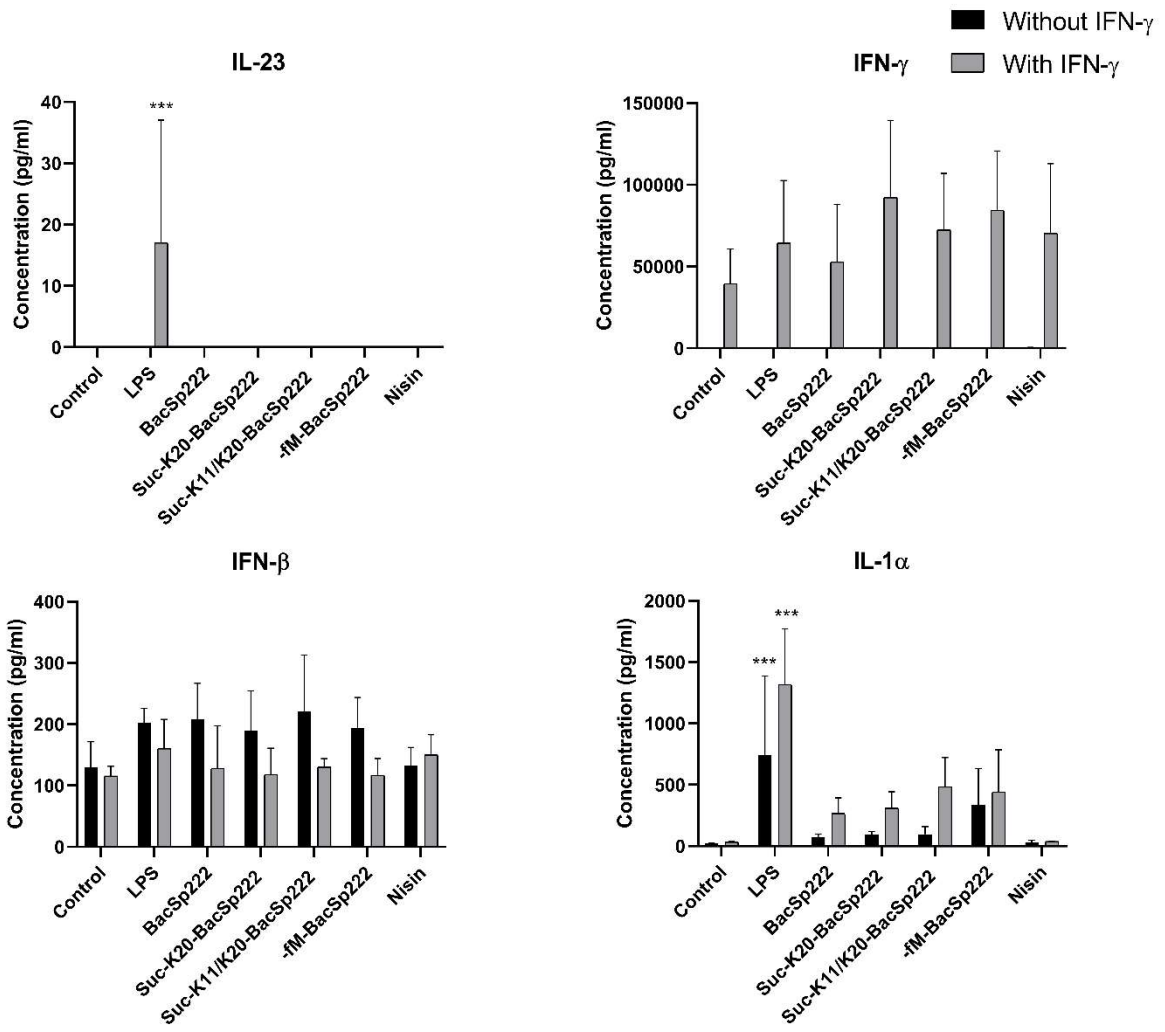
Supplementary Figure 8 The production of selected cytokines by P388.D1 cells exposed to various forms of bacteriocin BacSp222. The cells were incubated in medium (control), medium supplemented with LPS, BacSp222, suc-K20- BacSp222, suc-K11/K20- BacSp222, -fM-BacSp222 or nisin. After 24h the media were collected and subjected to flow cytometry analyses using LEGEND/Plex Mouse Inflammation Panel kit to determine cytokine concentrations. The bars represent the mean ± SEM (n=4). *** $p<0.001$ vs control, * $p<0.05$ vs control.

Abbreviations: LPS – lipopolysaccharide.



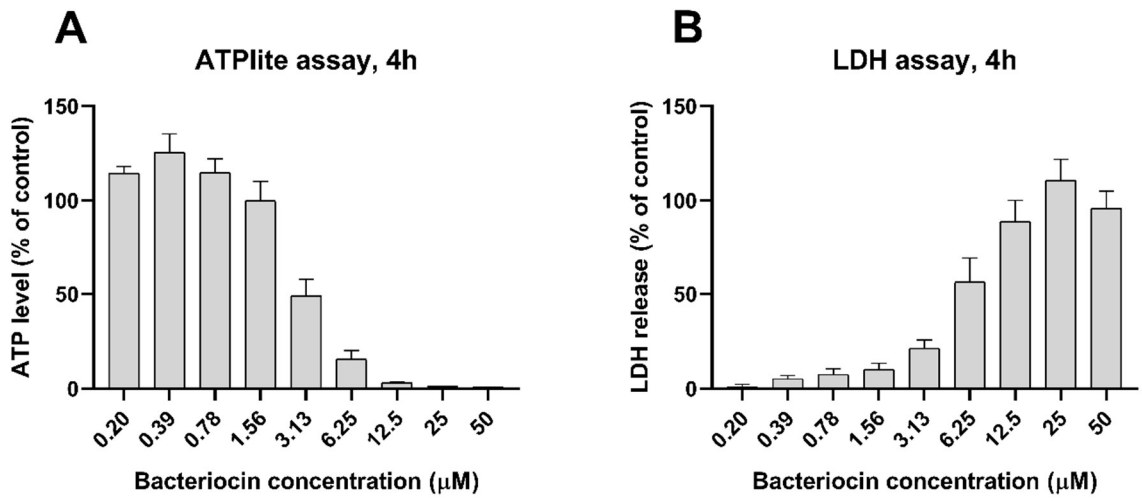
Supplementary Figure 9 The production of selected cytokines by P388.D1 cells exposed to various forms of bacteriocin BacSp222. The cells were incubated in medium (control), medium supplemented with LPS, BacSp222, suc-K20- BacSp222, suc-K11/K20- BacSp222, -fM-BacSp222 or nisin. After 24h the media were collected and subjected to flow cytometry analyses using LEGEND/Plex Mouse Inflammation Panel kit to determine cytokine concentrations. The bars represent the mean ± SEM (n=4). *** $p<0.001$ vs control, ** $p<0.01$ vs control.

Abbreviations: LPS – lipopolysaccharide.



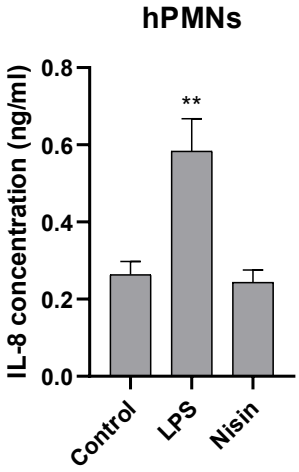
Supplementary Figure 10 The production of selected cytokines by P388.D1 cells exposed to various forms of bacteriocin BacSp222. The cells were incubated in medium (control), medium supplemented with LPS, BacSp222, suc-K20- BacSp222, suc-K11/K20- BacSp222, -fM-BacSp222 or nisin. After 24h the media were collected and subjected to flow cytometry analyses using LEGEND/Plex Mouse Inflammation Panel kit to determine cytokine concentrations. The bars represent the mean ± SEM (n=4). *** $p<0.001$ vs control.

Abbreviations: LPS – lipopolysaccharide.



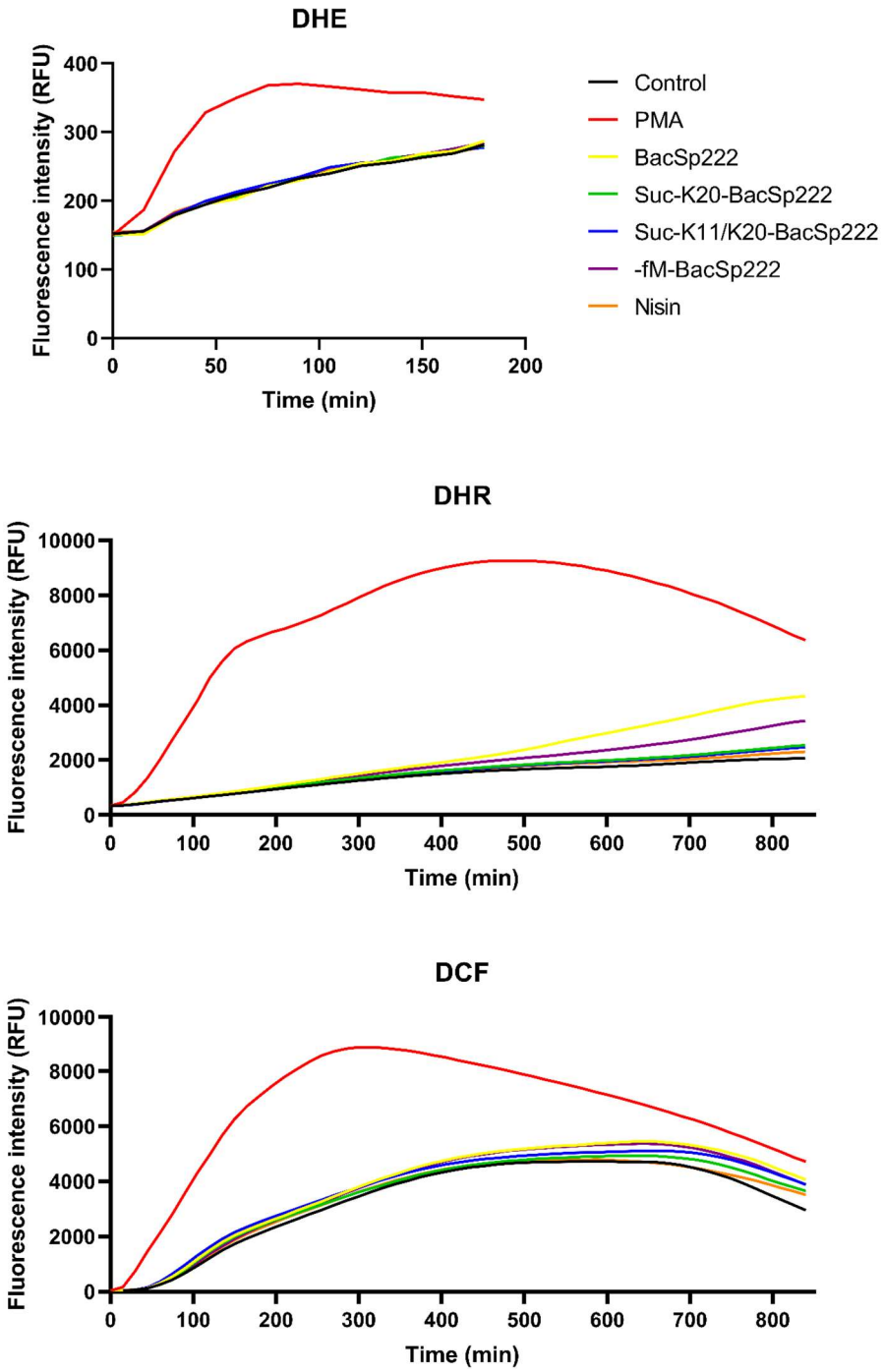
Supplemental Figure 11 The cytotoxicity effect of -fM-BacSp222 against human neutrophils.The cells were isolated from healthy volunteers' blood and exposed to various concentrations of -fM-BacSp222 for 4h. **A:** The viability of the cells was determined using an ATPlite assay. **B:** LDH activity in the post-cultured medium from cells stimulated with -fM-BacSp222. The bars represent the mean ± SEM (n=5).

Abbreviations: LDH - lactate dehydrogenase



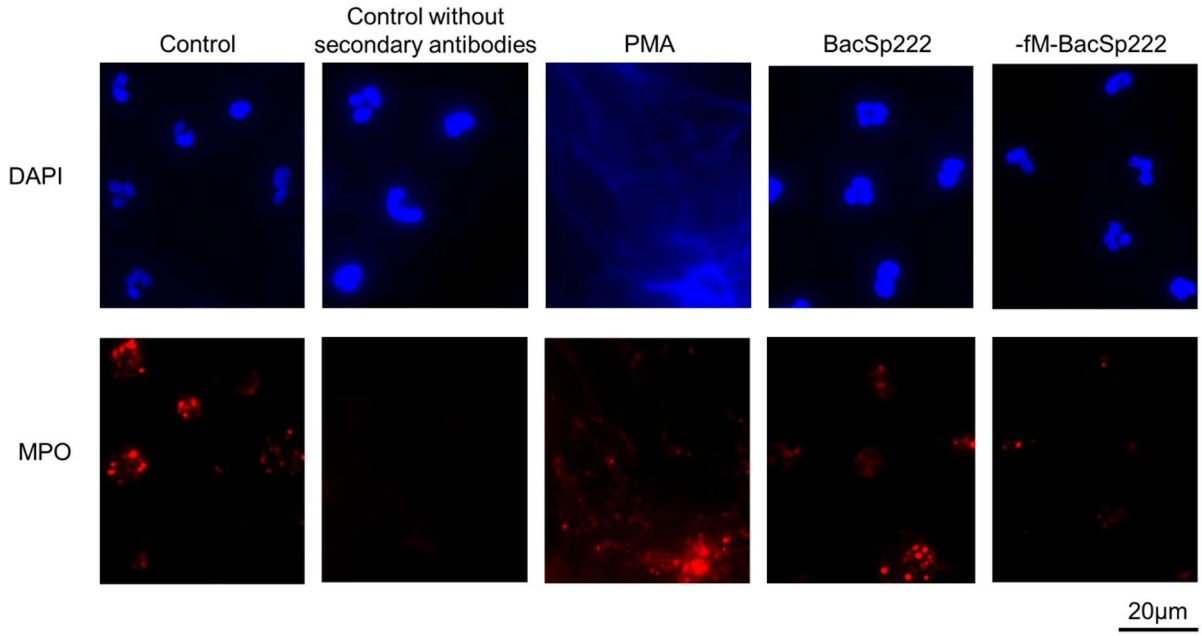
Supplementary Figure 12 Nisin does not stimulate IL-8 production by human PMNs. The cells were incubated overnight in control media or in media with LPS or nisin. The concentration of IL-8 in the culture media was determined by the ELISA assay. The bars represent the mean ± SEM (n=3).

Abbreviations: LPS – lipopolysaccharide. ** $p<0.01$ vs control.



Supplementary Figure 13 Analysis of ROS production by human PMNs exposed to the various forms of bacteriocins. Human PMNs were stained with the ROS probes: DHE, DHR, or DCF-HDA and exposed to PMA or bacteriocins. Fluorescence intensity corresponding to the level of intracellular ROS was measured for 3 or 14h, depending on the probe used (kinetic measurements).

Abbreviations: PMA - phorbol myristate acetate; PMNs - polymorphonuclear leukocytes; DHE- dihydroethidium; DHR - dihydrorhodamine; DCF- HDA - 2'-7'dichlorofluorescin diacetate; ROS - reactive oxygen species.



Supplementary Figure 14 Bacteriocins do not stimulate NETs formation by human PMNs. Human PMNs were incubated in medium (control), or in medium containing PMA, BacSp222, or -fM-BacSp222. Cellular DNA stained with DAPI (blue) and MPO stained using specific antibodies (red) were visualized using a fluorescence microscope.

Abbreviations: NETs - neutrophil extracellular traps; PMA - phorbol myristate acetate; PMNs - polymorphonuclear leukocytes; DAPI - 4,6-diamidino-2-phenyloindole; MPO - myeloperoxidase.