MiR-146b protects against pediatric pneumonia progression through MyD88/NF-κB signaling pathway

Lei Zhang
Zhengzhou Children's Hospital
Lili Dong
Zhengzhou Children's Hospital
Yu Tang
Zhengzhou Children's Hospital
Min Li
Zhengzhou Children's Hospital
Mingming Zhang (zhmm7777@yeah.net)
Zaozhuang Municipal Hospital https://orcid.org/0000-0002-9962-6382

Research article

Keywords: miR-146b; pediatric pneumonia; MyD88; NF κB pathway

Posted Date: July 26th, 2019

DOI: https://doi.org/10.21203/rs.2.11998/v1

Abstract

Background Pneumonia is a common respiratory disease worldwide that can be prevented and treated. However, it is considered to be the leading cause of children death. The present study was aimed to explore the role of miR-146b and its underlying mechanism in lipopolysaccharide (LPS)-induced inflammation injury in pediatric pneumonia. Methods Human fibroblasts WI-38 cells treated with LPS were subjected to construct cell model with inflammation injury. QRT-PCR or Western blot was applied to detect miR-146b and MyD88 expression. ELISA kit was used to analyze the production of pro-inflammatory factors. Cell viability was evaluated by CCK-8 assay. The apoptosis proteins and the downstream genes of NF-κB pathway were detected by Western blot. Results We displayed that miR-146b was downregulated, whereas MyD88 was upregulated in children with pneumonia and in WI-38 cells treated with LPS. Moreover, re-expression of miR-146b suppressed the production of inflammatory factors in the serum of pneumonia patients and WI-38 cells. Also, elevating miR-146b expression increased cell viability and reduced cell apoptosis. However, MyD88 overturned the protective effect of miR-146b on inflammation injury in pediatric pneumonia. Moreover, miR-146b overexpression inhibited the activation of NF-κB signaling pathway by suppressing MyD88. Conclusions These findings revealed that miR-146b attenuated the inflammation injury in pediatric pneumonia through inhibiting MyD88/NF-κB signaling pathway.

Background

Pneumonia has high morbidity and mortality, especially among children, resulting in 1.1 to 1.4 million child deaths per year (1, 2). Because of the high recurrence of pneumonia in children, it can cause a variety of serious complications and poor prognosis, which affects children's development and even leads to death (3, 4). Therefore, it is necessary to study the potential pathogenesis of pediatric pneumonia and determine the effective therapeutic targets.

MicroRNAs (miRNAs), a small non-coding RNA, regulate their target genes expression by binding 3'UTR (5). They are reported to take part in the process of cell proliferation, migration, apoptosis, as well as innate immunity, inflammation and infection (6). In the inflammation injury of pediatric pneumonia, previous studies have showed that numerous miRNAs played potential roles in its development. For instance, miR-141 displayed inhibitory effect on LPS-induced inflammation injury in WI-38 fibroblasts (7). Also, miR-1247 showed impeding effect on acute pneumonia (8). However, miR-194 and miR-20a revealed the promotion effect on inflammation injury in pediatric pneumonia (9, 10).

It turns out that miRNAs take part in tumors development and progression by targeting their mRNAs gene. For example, miR-146b targeted CCDC6 to modulate papillary thyroid cancer development (11). Also, FBXL10 was the direct target of miR-146b in regulating ovarian cancer cell proliferation, migration and invasion (12). Moreover, there were reports showed that miR-146b could regulate MyD88 expression in the proinflammatory response (13, 14). The involvement of MyD88 in inflammatory diseases has been extensively studied, such as ulcerative colitis, streptococcus pneumoniae meningitis and herpes simplex

encephalitis (15-17). Here, we aimed to investigate whether MyD88 was the target of miR-146b in regulating pediatric pneumonia progression and its underlying mechanism.

Myeloid differentiation factor 88 (MyD88) is a key anchoring protein of Toll-like receptor signaling pathway, which can lead phosphorylation of IκB inhibition and NF-κB activation and further induce inflammatory factor generation (18, 19). Previous studies showed that MyD88 played important roles in TLR4/MyD88/NF-κB signaling pathway and it was upregulated in inflammatory bowel disease (20). Here, we aimed to detect whether MyD88/NF-κB signaling pathway participated in pediatric pneumonia progression modulated by miR-146b.

Methods

Patients

A total of 20 children aged 8-15 years with pneumonia (10 females and 10 males) and 20 gender and age-matched healthy children with fever were enrolled in the study. The 40 patients were recruited from the emergency department of Zhengzhou Children's Hospital and they did not have any treatment before blood collection. We collected 5ml of peripheral venous blood from patients and obtained the serum via centrifugation. Parents of all the eligible patients accepted to participate in the study and gave informed written consent. The research process was reviewed and approved by Zhengzhou Children's Hospital and the ethic committee of Zhengzhou Children's Hospital.

Cell culture and cell transfection

The human WI-38 fibroblasts were obtained from American Type Culture Collection (ATCC, Manassas, USA). WI-38 cells were cultured in DMEM medium supplement with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin and maintained in a humidified incubator with 5% CO_2 atmosphere at 37°C. LPS (5 μg/mL) was then added into WI-38 cells for 12h to induce inflammation.

MiR-146b mimic or inhibitor was applied for increasing or decreasing miR-146b expression in WI-38 cells. MyD88 vector was used for overexpression of MyD88. The mimic/inhibitor, vector and their negative control were all purchased from GenePharma Co. (Shanghai, China). The transfection was performed for 48h with the help of Lipofectamine 3000 reagent (Invitrogen, Carlsbad, USA) according to the manufacturer's introduction.

Quantitative real time-PCR (QRT-PCR)

Total RNA was extracted using miRNeasy Mini Kit (Qiagen, Valencia, USA). Multiscribe RT kit and TaqMan MicroRNA Assay (Applied Biosystems, Foster, USA) were applied for performing reverse transcription and quantifying qRT-PCR, respectively. U6 and GAPDH were carried out for normalizing miR-146b expression and MyD88 mRNA expression, respectively. The follows were the primers sequences: miR-146b forward primer: 5'-TGACCCATCCTGGGCCTCAA-3', reverse primer: 5'-

CCAGTGGGCAAGATGTGGGCC-3’; U6 forward primer: 5’-CTCGCTTCGGCA GCACA-3’, reverse primer: 5’-AACGCTTCACGAATTTGCGT-3’; MyD88 forward primer: 5'-CTCCTCCACATCCTCCCTTC-3', reverse primer: 5'-GCTTGTGTCTCC AGTTGCC-3'; GAPDH forward primer: 5’-CTCGCTT CGGCAGCACA-3’, reverse primer: 5’-AACGCTTCACGAATTTGCGT-3’. All the data were analyzed through $2^{-\Delta\Delta CT}$ method.

Western blot

Proteins were extracted from cells using by RIPA buffer (Beyotime Biotechnology, Beijing, China). BCA kit was used for determining protein concentration. An equal amount of protein was resolved on SDS-PAGE, followed by transferred to PVDF membranes (Bio-Rad, Hercules, USA). After blocking with 5% skim milk for 1h at 37℃, the membranes were incubated with primary antibodies overnight at 4 ℃, subsequently, the secondary antibodies for 1h at 37 ℃. Finally, the enhanced chemiluminescence reagent was carried out for monitoring the bands. For ensuring equal protein loading, GAPDH was served as the internal control. ImageJ 1.49 (National Institute of Health, Bethesda, MD) was used to quantify the intensity of the bands.

Cell Counting Kit-8 (CCK-8) assay

CCK-8 assay was applied for detecting cell viability. The cells were cultured in 96-well plates for 24h. Then, CCK-8 solution (10 μL) was added into each well and incubated for another 2h. Finally, the absorbance at 450 nm was detected by microplate reader (Bio-Tek, Winooski, USA). Cell viability was evaluated by the absorbance.

Enzyme-linked immunosorbent assay (ELISA)

The inflammatory cytokines in supernatant serum samples of children with pneumonia and in cell culture supernatant treated with LPS were quantified by corresponding ELISA kits (R&D Systems, Inc., Minneapolis, USA) following the manufacturer's protocols, including interleukin (IL)-6, tumor necrosis factor (TNF)-α and interleukin (IL)-1β ELISA Kits. Then, a microplate reader was applied for measuring the absorbance at 450 nm. The concentrations of IL-6, TNF-α and IL-1β were normalized and calculated based on the linear calibration curves obtained by standard solutions.

Luciferase reporter assay

The fragments of MyD88 3’UTR containing the wild-type (WT) or mutated binding sites of miR-146b were inserted into psiCHECK-2 vector (Promega, Madison, WI). Then, cells were co-transfected with luciferase reporter vectors and miR-146b mimic with the aid of Lipofectamine 2000. After transfection for 24h, the luciferase activities were analyzed by a luciferase reporter assay system.

Statistical analysis

All experiments were repeated in triplicates. The values were represented as mean ± SD and GraphPad Prism 6.0 software was applied for performing statistical analyses. The one-way Analysis of Variance

(ANOVA) or student t-test was used to calculate P values between different groups. P value of <0.05 was considered as statistically significant.

Results

Down-regulation of miR-146b and up-regulation of MyD88 in children patients with pneumonia

To investigate the role of miR-146b in pediatric pneumonia progression, we first detected its expression in the serum of children patients with pneumonia. As Figure 1A displayed that miR-146b was decreased in patients with pneumonia compared with the healthy children. Also, we measured MyD88 expression in pneumonia patients and results showed that MyD88 was increased in patients with pneumonia compared with the healthy children (Figure 1B). Moreover, the production of inflammatory cytokines (IL-6, TNF-α, IL-1β) was increased significantly in pneumonia patients in comparison with healthy controls by ELISA kit (Figure 1C-1E). These findings revealed that miR-146b and MyD88 might play important effect on inflammation injury in pediatric pneumonia.

MiR-146b attenuated the inflammation injury in pediatric pneumonia

Next, we explored miR-146b effect on pediatric pneumonia progression *in vitro.* WI-38 fibroblasts treated with LPS were used to induce inflammation injury. Then, we detected miR-146b expression by qRT-PCR. As we saw in Figure 2A, miR-146b expressional level was decreased in LPS-injured WI-38 cells. Afterwards, miR-146b was increased by miR-146b mimic and decreased by miR-146b inhibitor (Figure 2B). CCK-8 assay displayed that cell viability was suppressed after WI-38 cells were treated with LPS. However, treatment with miR-146b mimic enhanced, while miR-146b inhibitor repressed WI-38 cell viability (Figure 2C). For cell apoptosis, miR-146b showed the opposite effect (Figure 2D). Furthermore, ELISA analysis displayed that miR-146b mimic inhibited the production of IL-6, TNF-α and IL-1β induced by LPS in WI-38 cells, whereas miR-146b inhibitor facilitated their production (Figure 2E-2G). These data indicated that miR-146b protected WI-38 cells from inflammatory injury.

MyD88 was the direct target of miR-146b

To explore the underlying mechanism of miR-146b in regulating pediatric pneumonia progression, we first determined the direct target of miR-146b. As Figure 3A shown, MyD88 was the possible target of miR-146b predicted by TargetScanHuman 7.2. Furthermore, luciferase reporter assay was applied for further confirming whether MyD88 was the direct target of miR-146b. As we saw in Figure 3B, the relative luciferase activity was decreased significantly by miR-146b mimic in WT, while there have no changes in MuT. These results revealed that MyD88 was the direct target of miR-146b. Moreover, we detected the relationship between miR-146b and MyD88 expression. QRT-PCR and Western Blot results displayed that miR-146b up-regulation repressed MyD88 expression both in mRNA and protein level, while miR-146b down-regulation promoted MyD88 expression (Figure 3C-3D). In addition, regression analysis revealed that the correlation of miR-146b with MyD88 was negatively (Figure 3E). These findings suggested that miR-146b modulated pediatric pneumonia progression by negatively regulating MyD88.

MyD88 overturned miR-146b effect on pediatric pneumonia progression

To investigate MyD88 effect on pediatric pneumonia progression regulated by miR-146b, we first measured MyD88 expression in WI-38 cells treated with LPS. As Figure 4A shown, MyD88 was increased in LPS-injured WI-38 cells. Then, MyD88 expression was increased by over-expression of MyD88 (Figure 4B). Afterwards, MyD88 effect on cell viability, apoptosis and inflammatory cytokines was detected by CCK-8 assay, Western blot assay and ELISA assay. Results displayed that overexpression of MyD88 reversed miR-146b suppression effect on cell apoptosis (Figure 4C), but attenuated miR-146b promotion effect on cell viability (Figure 4D). For inflammatory cytokines, MyD88 up-regulation overturned miR-146b inhibitory effect on the production of IL-6, TNF-α and IL-1β (Figure 4E-4G). These findings indicated that MyD88 could reverse miR-146b protecting effect against inflammatory injury in pediatric pneumonia.

MiR-146b regulated NF-κB signaling pathway by suppressing MyD88

To explore whether NF-κB signaling pathway was involved in the progression of pediatric pneumonia, we detected the downstream genes of NF-κB pathway regulated by miR-146b via Western blot *in vitro* (Figure 5A). Results showed that the phosphorylation of p65 was activated by LPS-treated in WI-38 cells, and overexpression of miR-146b inhibited p-p65 expression, while increasing both MyD88 and miR-146b expression promoted p-p65 expression (Figure 5B). However, the phosphorylation of IkBα was inhibited by LPS-treated in WI-38 cells, and overexpression of miR-146b facilitated p-IkBα expression, while increasing both MyD88 and miR-146b expression suppressed p-IkBα expression (Figure 5C). These results displayed that miR-146b modulated NF-κB signaling pathway by suppressing MyD88.

Discussion

Mounting evidences have reported that miRNAs play important roles in inflammatory diseases, including pediatric pneumonia. The present study stated that miR-146b expression was decreased in WI-38 cells after administrated with LPS and it was negatively correlated with MyD88 expression. These findings made us speculate that miR-146b/MyD88 axis might be involved in inflammation response of pediatric pneumonia. Thus, we aimed to survey miR-146b/MyD88 role in the progression of pediatric pneumonia *in vivo and in vitro*.

In fact, there are abundant evidences that miR-146b is a tumor regulator and associated with a variety of autoimmune diseases, such as ulcerative colitis and rheumatoid arthritis (21, 22). However, little is known about miR-146b expression in pulmonary inflammation, and it is not fully clear whether the abnormal expression of miR-146b in pulmonary inflammation is associated with inflammatory damage. Previous studies have reported that miR-146b played important role in inflammatory bowel disease (23). Zhang L et al found that miR-146b was down-regulated in chronic nonbacterial prostatitis and might be a effective target for patient treatment (24). Moreover, miR-146b participated in LPS-induced inflammation and apoptosis in murine chondrogenic ATDC5 cells (25). In our study, we displayed that miR-146b up-regulation attenuated pediatric pneumonia progression through facilitating cell viability, suppressing cell apoptosis, decreasing production of IL-6, TNF-α and IL-1β.

MyD88 is a key linker molecule in the Toll-like receptor (TLR) signaling pathway and plays an important role in the disease development. MyD88 was proved to be involved in inflammatory lung injury, including pneumonia. For instance, Wang X found that MyD88 contributed to ventilation-induced lung injury and inflammation (26). Also, Sônego F et al displayed that MyD88 took part in P. aeruginosa-induced pulmonary infection (27). Here, we revealed that MyD88 played important role in the progression of pediatric pneumonia regulated by miR-146b. Moreover, we exhibited that MyD88 was the direct target of miR-146b.

It has been reported that in inflammatory diseases, the release of inflammatory mediators can activate a variety of intracellular signaling pathways, including the NF-κB signal pathway (28). In the present study, we showed that treatment WI-38 cells with LPS promoted the activation of NF-κB signaling pathways, which was blocked by miR-146b mimic and then activated after overexpression of MyD88.

Conclusion

In conclusion, the findings implied that miR-146b protected against pediatric pneumonia progression by inhibiting MyD88 expression and then suppressed MyD88-regulated NF-κB signaling pathway.

Abbreviations

LPS: Lipopolysaccharide **MyD88:** Myeloid differentiation factor 88 **ATCC:** American Type Culture Collection **FBS:** Fetal Bovine Serum **QRT-PCR:** Quantitative real time-PCR **ELISA:** Enzyme-linked immunosorbent assay **IL-6:** interleukin-6 **TNF-α:** tumor necrosis factor-α **IL-1β:** interleukin-1β **ANOVA:** Analysis of Variance **TLR:** Toll-like receptor

Declarations

Acknowledgements

None

Authors' contributions

LZ as the first author contributed significantly to manuscript preparation. LD, YT and ML performed the data analyses and interpretation of data. MZ as the corresponding author contributed to the conception of the study. All authors read and approved the final manuscript.

Funding

This research did not receive any funding from agencies in the public, commercial, or not-for-profit sectors.

Availability of data and materials

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

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Zaozhuang Municipal Hospital. Signed written informed consents were obtained from either the parent(s) or legal guardian(s) of each study participant as well as from the children aged >8 years

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflict interest statements.

References

1. L. Liu, H. L. Johnson, S. Cousens, et al. Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. Lancet. 2012;379(9832):2151-61. 2. C. H. Chen, H. J. Wen, P. C. Chen, et al. Prenatal and postnatal risk factors for infantile pneumonia in a representative birth cohort. Epidemiol Infect. 2012;140(7):1277-85. 3. J. K. Leyenaar, T. Lagu, M. S. Shieh, P. S. Pekow, P. K. Lindenauer. Management and outcomes of pneumonia among children with complex chronic conditions. Pediatr Infect Dis J. 2014;33(9):907-11. 4. Z. A. Bhutta, J. K. Das, N. Walker, et al. Interventions to address deaths from childhood pneumonia and diarrhoea equitably: what works and at what cost? Lancet. 2013;381(9875):1417-29. 5. D. P. Bartel. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004;116(2):281-97. 6. W. P. Kloosterman, R. H. Plasterk. The diverse functions of microRNAs in animal development and disease. Dev Cell. 2006;11(4):441-50. 7. B. Quan, H. Zhang, R. Xue. miR-141 alleviates LPS-induced inflammation injury in WI-38 fibroblasts by up-regulation of NOX2. Life Sci. 2019;216:271-8. 8. J. Guo, Y. Cheng. MicroRNA-1247 inhibits lipopolysaccharides-induced acute pneumonia in A549 cells via targeting CC chemokine ligand 16. Biomed Pharmacother. 2018;104:60-8. 9. F. Xie, L. Yang, L. Han, B. Yue. MicroRNA-194 Regulates Lipopolysaccharide-Induced Cell Viability by Inactivation of Nuclear Factor-kappa B Pathway. Cell Physiol Biochem. 2017;43(6):2470-8. 10. Z. Liu, H. Yu, Q. Guo. MicroRNA20a promotes inflammation via the nuclear factorkappaB signaling pathway in pediatric pneumonia. Mol Med Rep. 2018;17(1):612-7. 11. M. Jia, Y. Shi, Z. Li, X. Lu, J. Wang. MicroRNA-146b-5p as an oncomiR promotes papillary thyroid carcinoma development by targeting CCDC6. Cancer Lett. 2019;443:145-56. 12. M. Yan, X. Yang, R. Shen, et al. miR-146b promotes cell proliferation and increases chemosensitivity, but attenuates cell migration and invasion via FBXL10 in ovarian cancer. Cell Death Dis. 2018;9(11):1123. 13. Z. Wang, L. R. Filgueiras, S. Wang, et al. Leukotriene B4 enhances the generation of proinflammatory microRNAs to promote MyD88-dependent macrophage

activation. J Immunol. 2014;192(5):2349-56. 14. A. Ordas, Z. Kanwal, V. Lindenberg, et al. MicroRNA-146 function in the innate immune transcriptome response of zebrafish embryos to Salmonella typhimurium infection. BMC Genomics. 2013;14:696. 15. J. P. Wang, L. N. Dong, M. Wang, J. Guo, Y. Q. Zhao. MiR-146a regulates the development of ulcerative colitis via mediating the TLR4/MyD88/NF-kappaB signaling pathway. Eur Rev Med Pharmacol Sci. 2019;23(5):2151-7. 16. Y. Zhang, Y. Jiang, D. Lu. Diosmetin Suppresses Neuronal Apoptosis and Inflammation by Modulating the Phosphoinositide 3-Kinase (PI3K)/AKT/Nuclear Factor-kappaB (NF-kappaB) Signaling Pathway in a Rat Model of Pneumococcal Meningitis. Med Sci Monit. 2019;25:2238-45. 17. M. Mancini, G. Caignard, B. Charbonneau, et al. Rel-Dependent Immune and Central Nervous System Mechanisms Control Viral Replication and Inflammation during Mouse Herpes Simplex Encephalitis. J Immunol. 2019;202(5):1479-93. 18. L. A. O'Neill. The role of MyD88-like adapters in Toll-like receptor signal transduction. Biochem Soc Trans. 2003;31(Pt 3):643-7. 19. I. T. Lee, C. W. Lee, W. H. Tung, et al. Cooperation of TLR2 with MyD88, PI3K, and Rac1 in lipoteichoic acid-induced cPLA2/COX-2-dependent airway inflammatory responses. Am J Pathol. 2010;176(4):1671-84. 20. T. A. Reaves, A. C. Chin, C. A. Parkos. Neutrophil transepithelial migration: role of toll-like receptors in mucosal inflammation. Mem Inst Oswaldo Cruz. 2005;100 Suppl 1:191-8. 21. F. Deng, S. He, S. Cui, et al. A Molecular Targeted Immunotherapeutic Strategy for Ulcerative Colitis via Dual-targeting Nanoparticles Delivering miR-146b to Intestinal Macrophages. J Crohns Colitis. 2019;13(4):482-94. 22. E. Zilahi, T. Tarr, G. Papp, Z. Griger, S. Sipka, M. Zeher. Increased microRNA-146a/b, TRAF6 gene and decreased IRAK1 gene expressions in the peripheral mononuclear cells of patients with Sjogren's syndrome. Immunol Lett. 2012;141(2):165-8. 23. P. Chen, Y. Li, L. Li, et al. Circulating microRNA146b-5p is superior to C-reactive protein as a novel biomarker for monitoring inflammatory bowel disease. Aliment Pharmacol Ther. 2019;49(6):733-43. 24. L. Zhang, Y. Liu, X. G. Chen, et al. MicroRNA expression profile in chronic nonbacterial prostatitis revealed by next-generation small RNA sequencing. Asian J Androl. 2018. 25. H. Jin, H. Zhang, T. Ma, et al. Resveratrol Protects Murine Chondrogenic ATDC5 Cells Against LPS-Induced Inflammatory Injury Through Up-Regulating MiR-146b. Cell Physiol Biochem. 2018;47(3):972-80. 26. X. Wang, B. Luo, Y. Lu, et al. The triggering receptor expressed by myeloid cells-1 activates TLR4-MyD88-NF-kappaB-dependent signaling to aggravate ventilation-induced lung inflammation and injury in mice. Cell Tissue Res. 2018;374(1):137-48. 27. F. Sonego, F. V. S. Castanheira, C. V. Horta, et al. The host control of a clinical isolate strain of P. aeruginosa infection is independent of Nod-1 but depends on MyD88. Inflamm Res. 2018;67(5):435-43. 28. J. Wang, Y. T. Liu, L. Xiao, L. Zhu, Q. Wang, T. Yan. Anti-inflammatory effects of apigenin in lipopolysaccharide-induced inflammatory in acute lung injury by suppressing COX-2 and NF-kB pathway. Inflammation. 2014;37(6):2085-90.

Figures

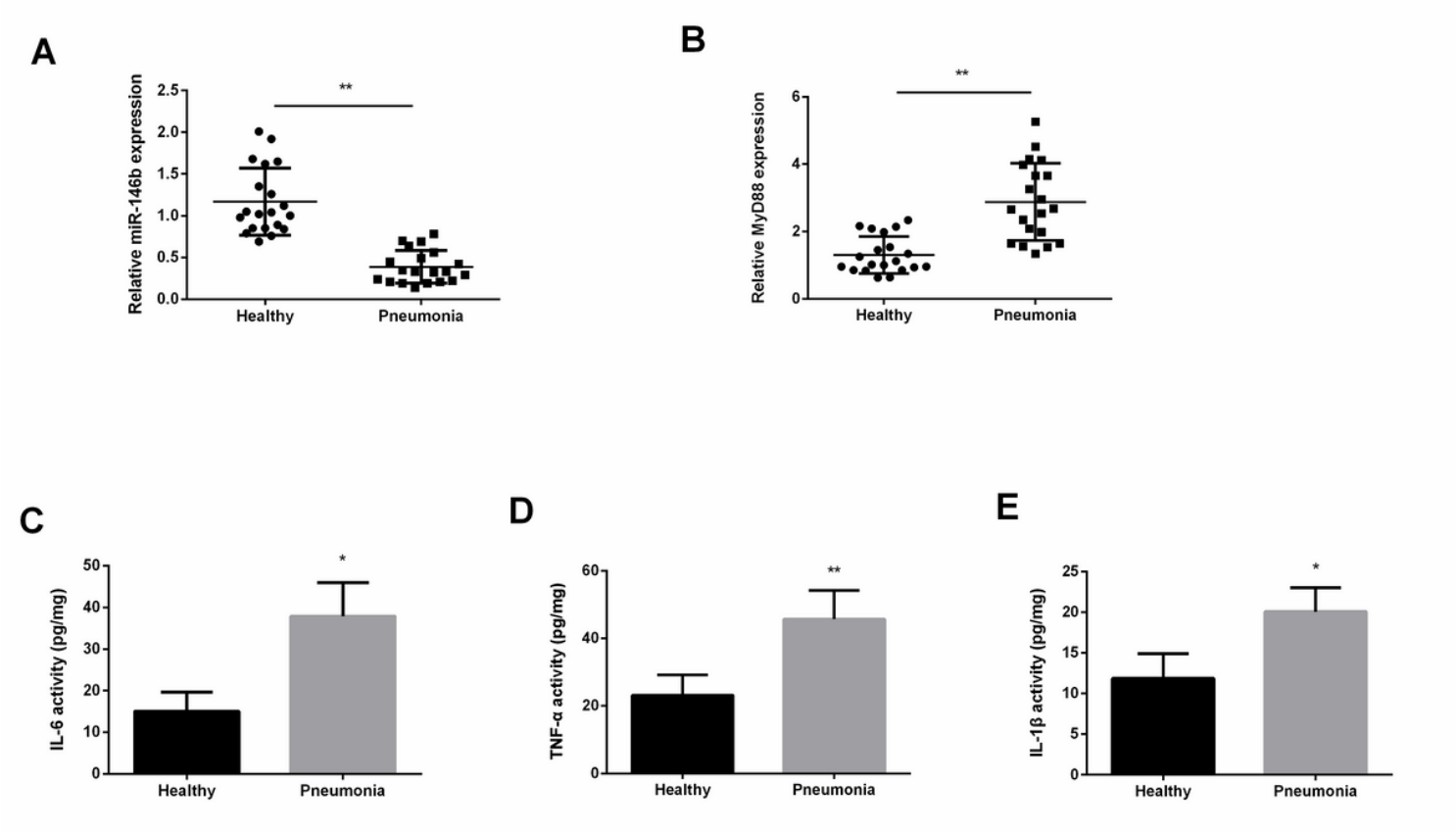

Figure 1

Down-regulation of miR-146 and up-regulation of MyD88 in children patients with pneumonia. (A) miR-146b expression measured in children with pneumonia and healthy children by qRT-PCR. (B) MyD88 expression measured in children with pneumonia and healthy children by qRT-PCR. (C) IL-6 production detected in children with pneumonia and healthy children by ELISA. (D) TNF-α production detected in children with pneumonia and healthy children by ELISA. (E) IL-1β production detected in children with pneumonia and healthy children by ELISA (n=20). *P<0.05, **P<0.01

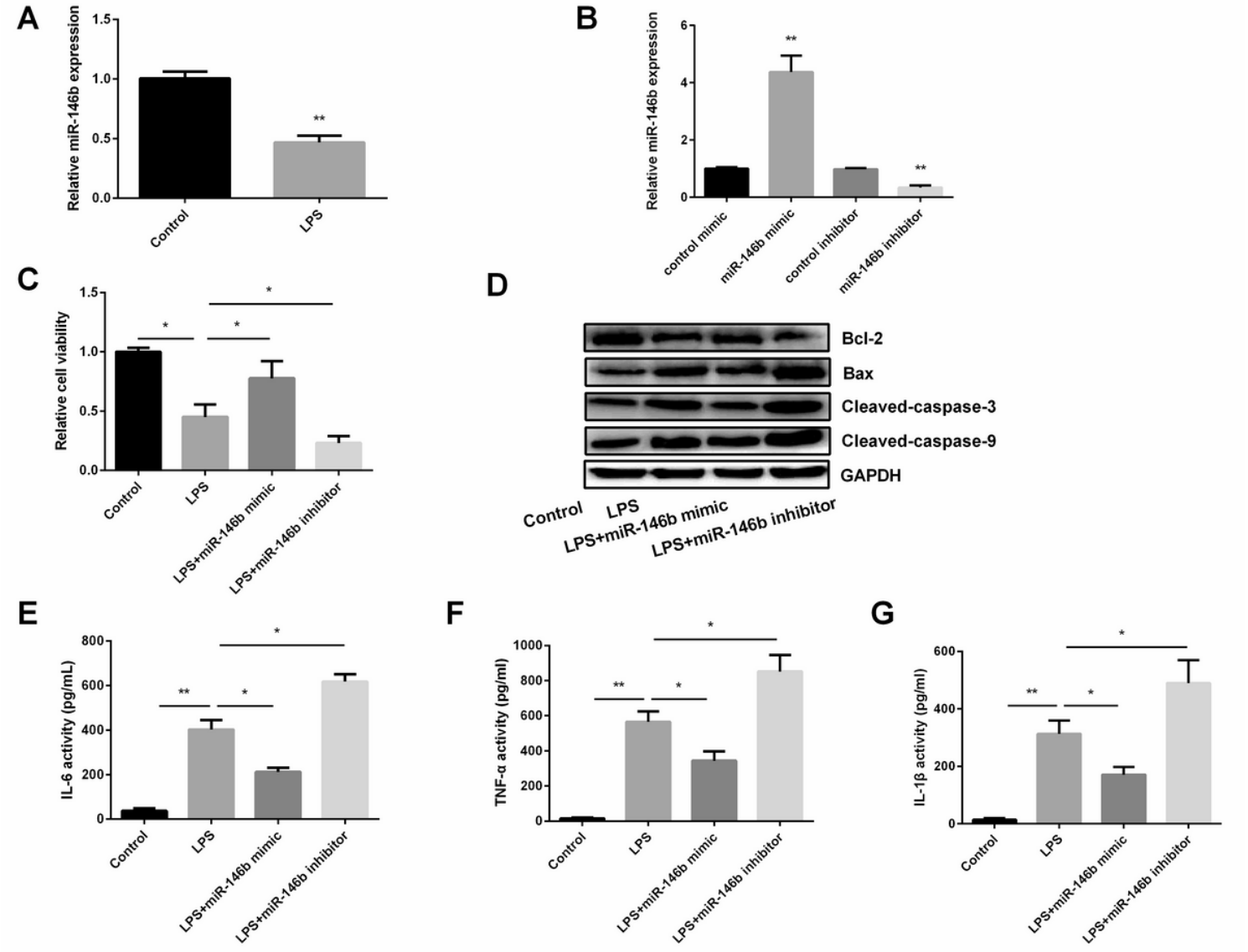

Figure 2

Inhibitory effect of miR-146b on pediatric pneumonia progression. (A) Detection of miR-146b expression in LPS-injured WI-38 cells. (B) Detection of miR-146b expression in WI-38 cells after increasing or decreasing miR-146b. (C) Measurement of WI-38 cell viability in LPS-injured WI-38 cells after transfection with miR-146b mimic or inhibitor. (D) Measurement of Bcl-2, Bax, Cleaved-caspase-3 and Cleaved-caspase-9 protein levels in LPS-injured WI-38 cells after transfection with miR-146b mimic or inhibitor. (E) Measurement of IL-6, TNF α (F) and IL 1β (G) production in LPS-injured WI-38 cells after transfection with miR-146b mimic or inhibitor. *P<0.05, **P<0.01

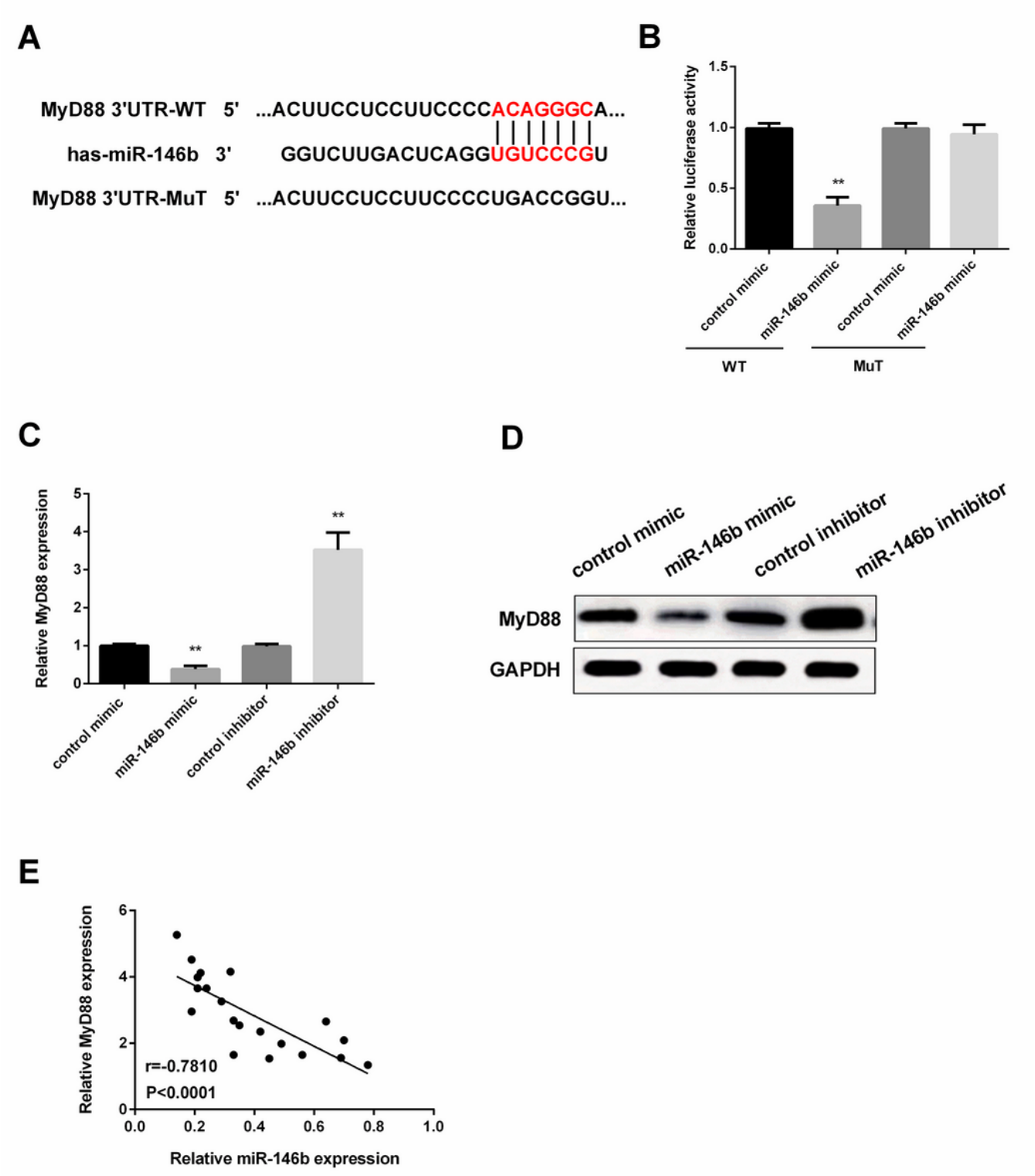

Figure 3

MyD88 was the direct target of miR-146b. (A) The prediction binding sites of miR-146b and MyD88. (B) Relative luciferase activity tested in WI-38 cells after treated with miR-146b mimic. (C) Relative mRNA expression and protein level (D) of MyD88 in WI-38 cells after treated with miR-146b mimic or inhibitor. (E) The relationship between miR-146b and MyD88 expression. *P<0.05, **P<0.01

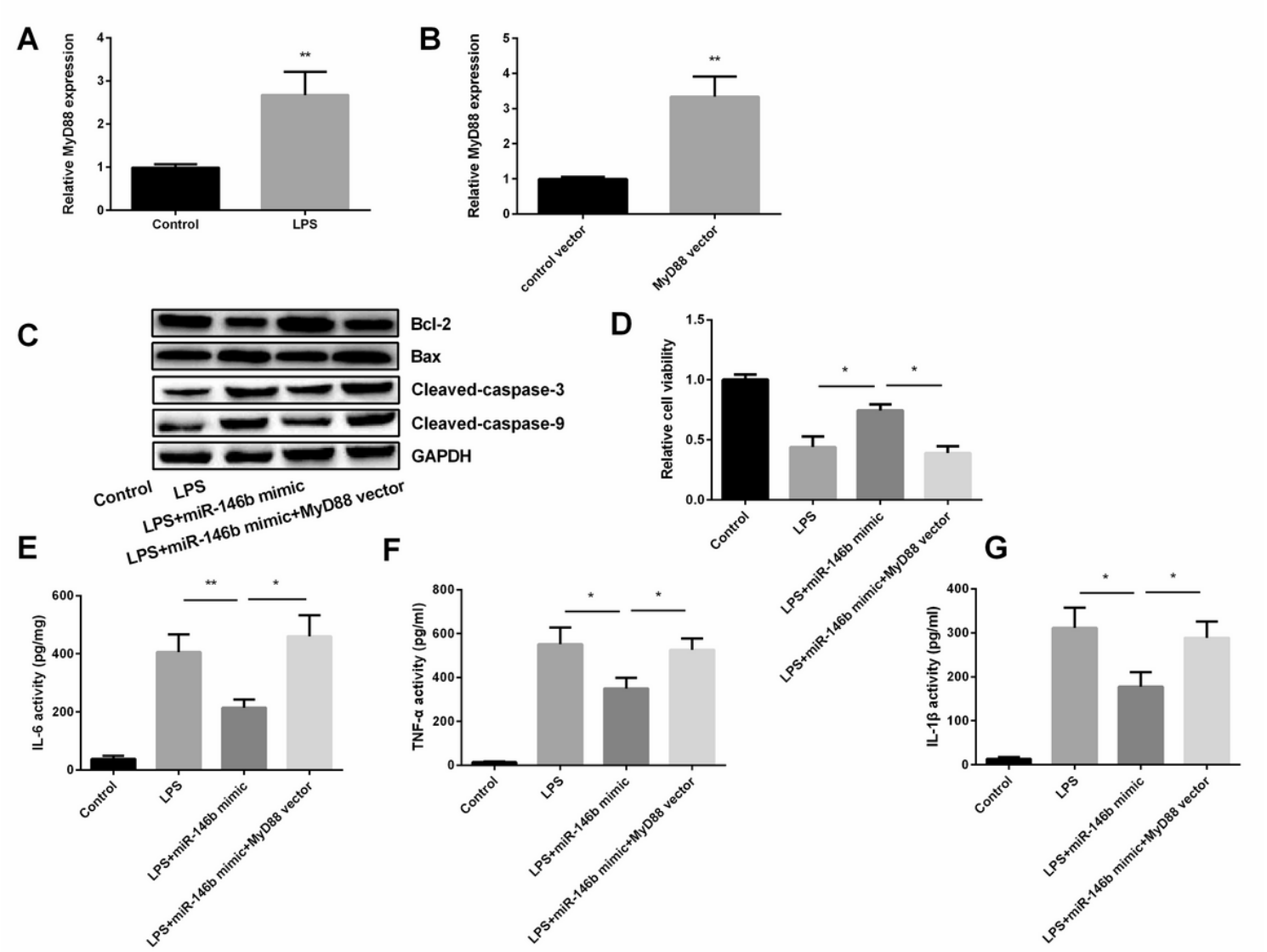

Figure 4

Reversion effect of MyD88 on pediatric pneumonia progression regulated by miR-146b. (A) MyD88 expression detected in LPS-injured WI-38 cells by qRT-PCR. (B) MyD88 expression detected in WI-38 cells after overexpression of MyD88. (C) Bcl-2, Bax, Cleaved-caspase-3 and Cleaved-caspase-9 protein levels detected in LPS-injured WI-38 cells after treated with miR-146b mimic or miR-146b mimic and MyD88 vector. (D) Cell viability detected in LPS-injured WI-38 cells after overexpression of miR-146b alone or both miR-146b and MyD88. (E) IL-6, TNF α (F) and IL 1β (G) production measured in LPS-injured WI-38 cells after treated with miR-146b mimic or both miR-146b mimic and MyD88 vector. *P<0.05, **P<0.01

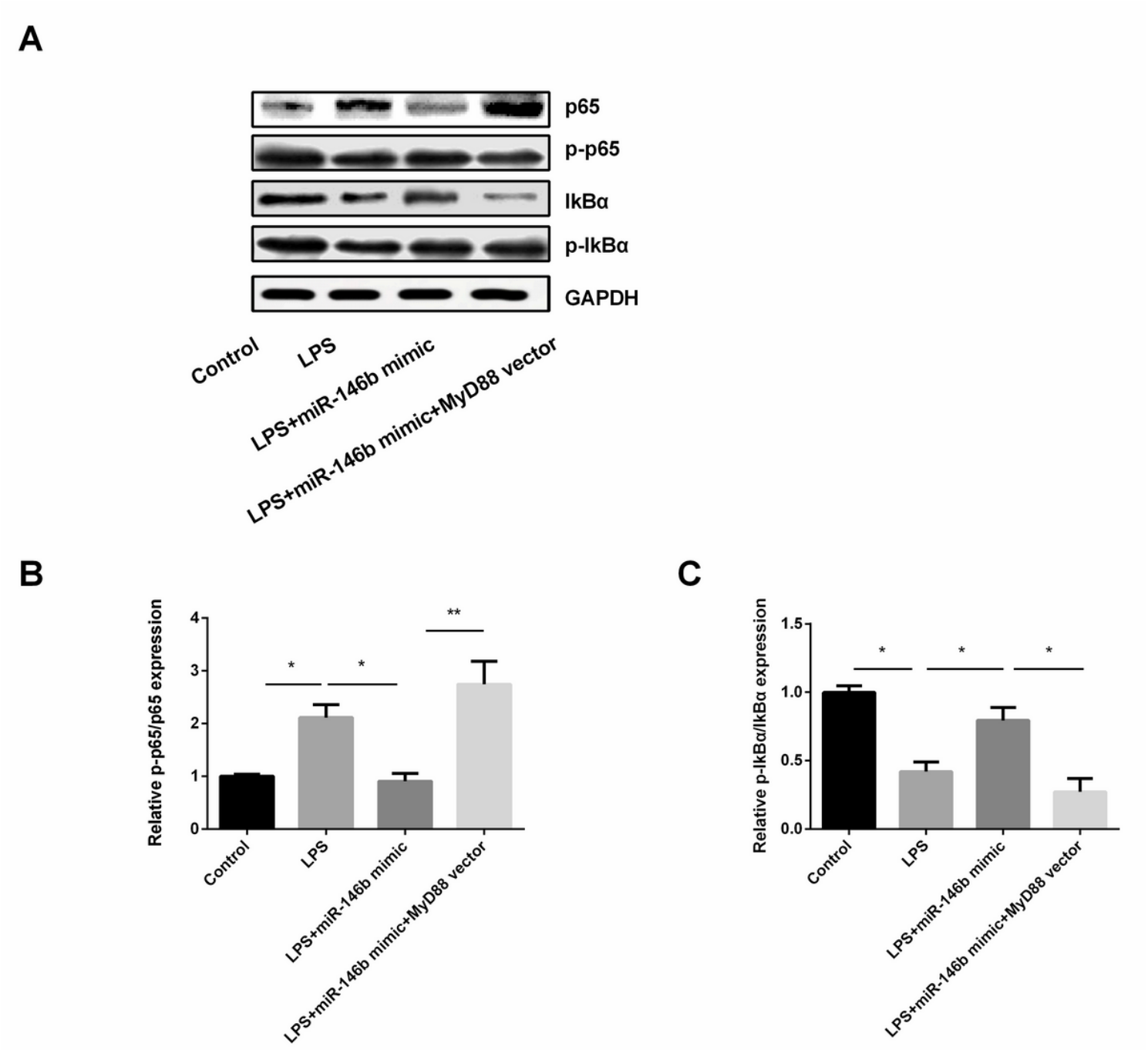

Figure 5

Inhibitory effect of miR-146b on the activation of NF κB signaling pathway. (A) Western blot analysis of p65, p-p65 and IkBα, p-IkBα level in LPS-injured WI-38 cells after transfection with miR-146b mimic or both miR-146b mimic and MyD88 vector. (B) Quantitative of p-p65/p65 and p-IkBα/IkBα (C) relative level in LPS-injured WI-38 cells after transfection with miR-146b mimic or both miR-146b mimic and MyD88 vector. *P<0.05, **P<0.01