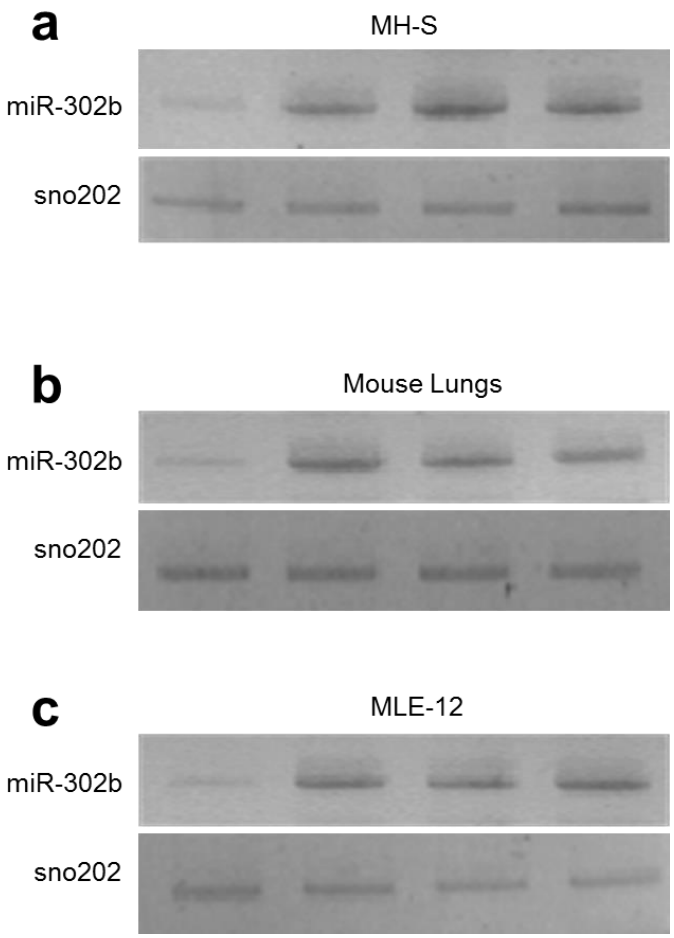
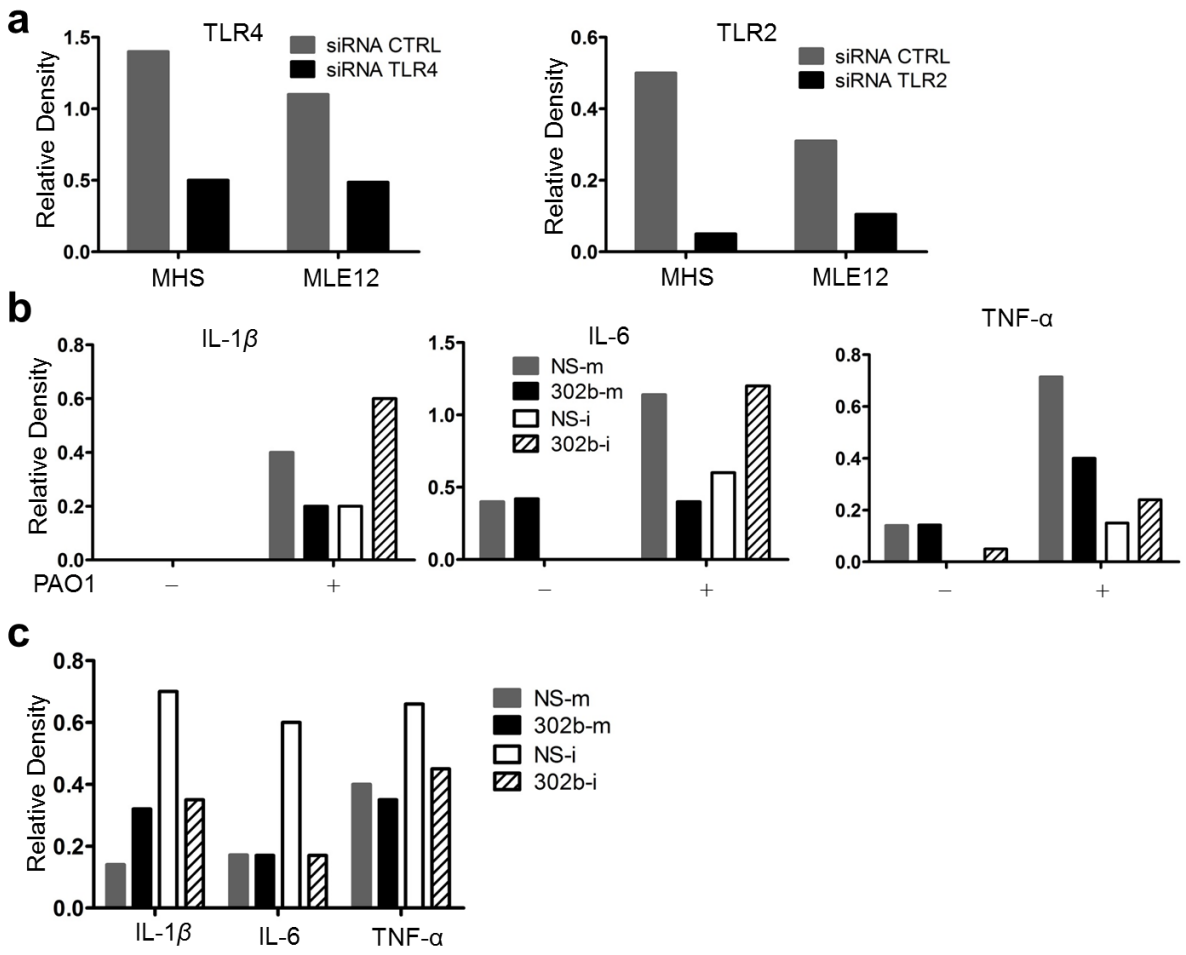
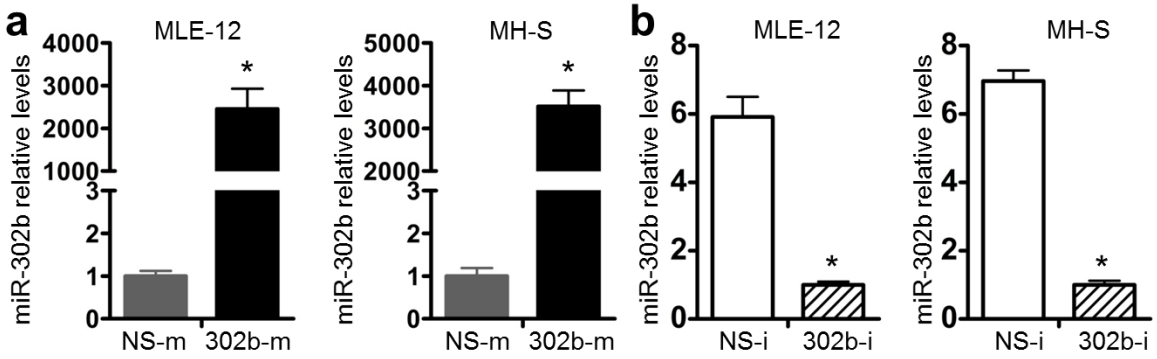


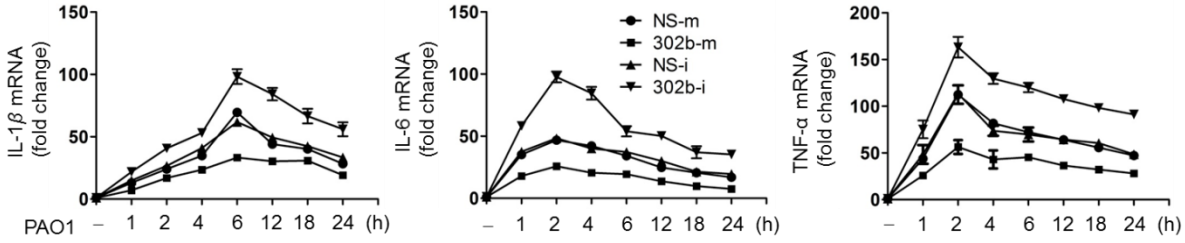
Supplementary Figure 1 | Validate the expression of miR-302b after bacterial infection by northern blot. Northern blot analysis of miR-302b expression following infection with PAO1, PAK and Kp in (**A**) lung tissues from the infected mice, (**B**) MLE-12 cells and (**C**) MH-S cells. These data are representative of three experiments.



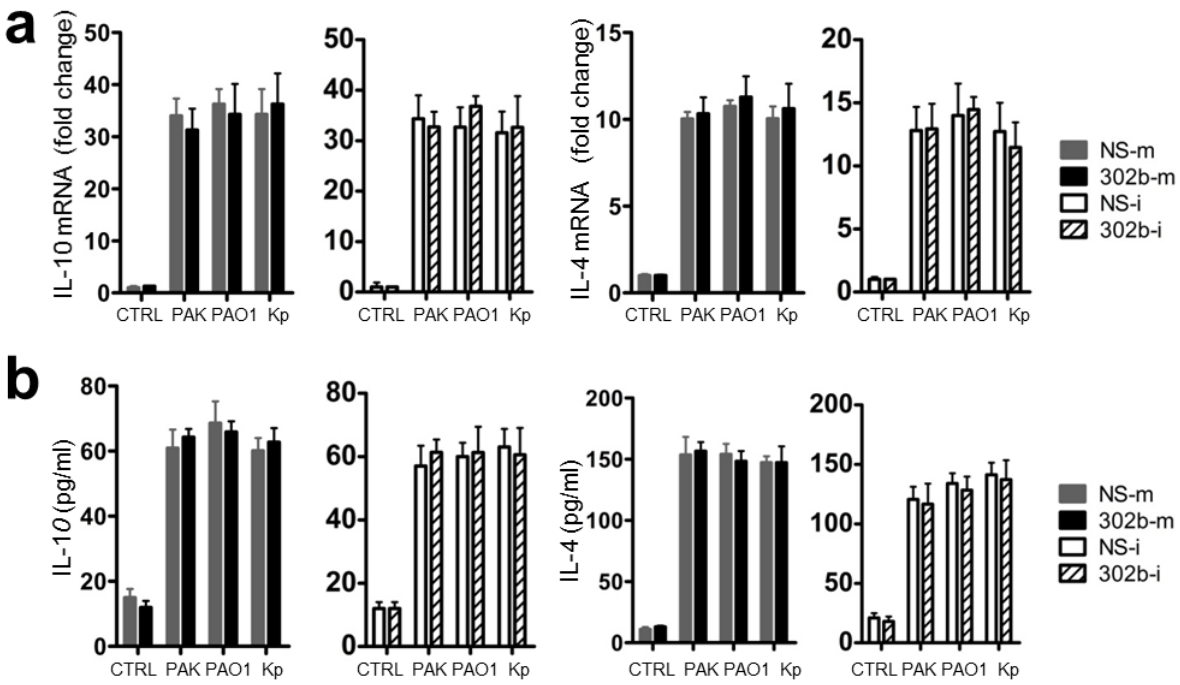
Supplementary Figure 2 | Validate the function of miR-302b mimics and miR-302b inhibitor. Real-time qPCR analysis of miR-302b expression in MLE-12 and MH-S cells transfected with (**A**) miRNA mimic negative control (NS-m), miR-302b mimics (302b-m), (**B**) miRNA inhibitor negative control (NS-i), and miR-302b inhibitor (302b-i). These data are representative of three experiments and are shown as means.



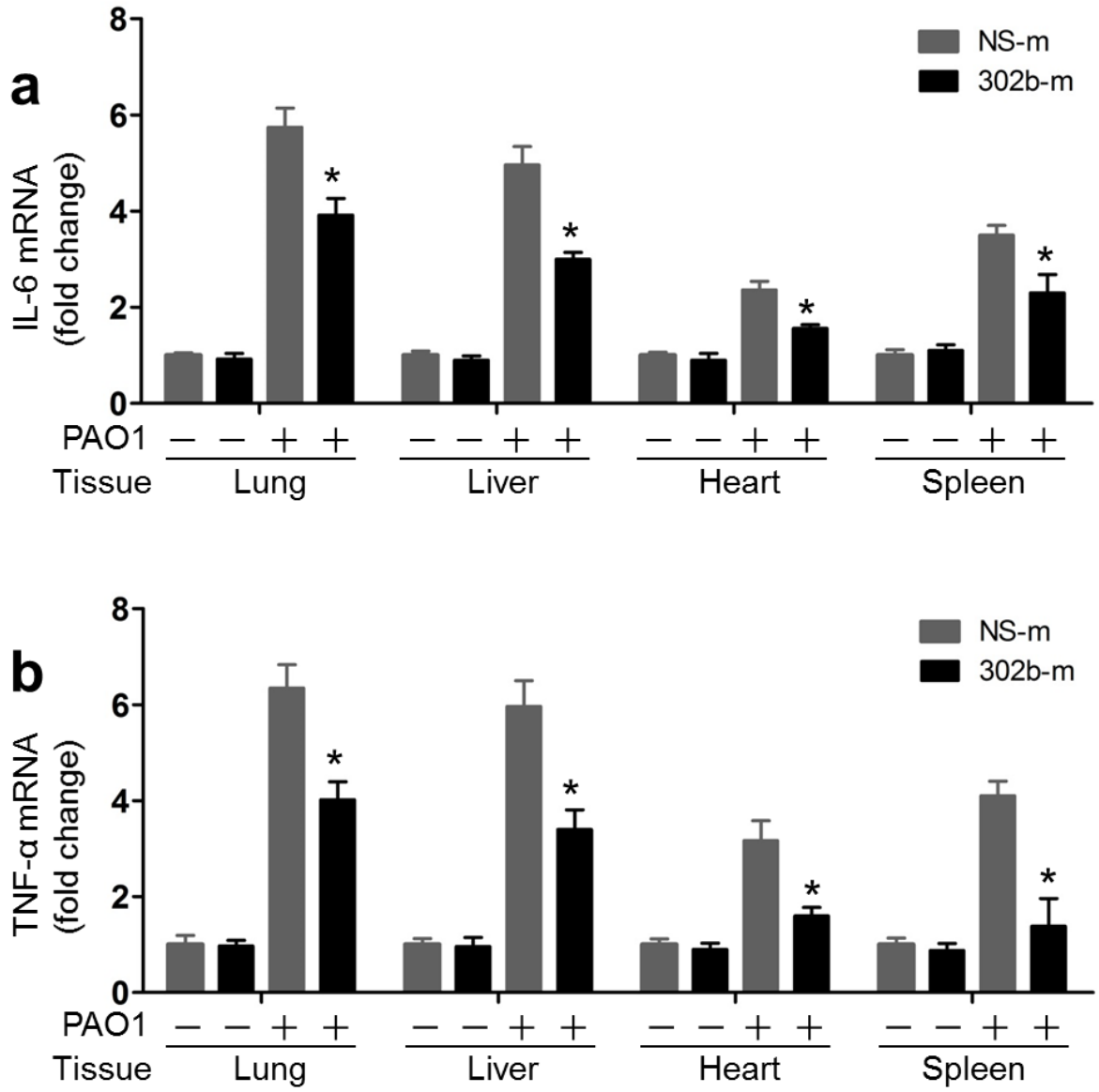
Supplementary Figure 3 | Densitometric quantification of the western blotting gel data presented in Fig. 2, 3 and 4 (in text) using Quantity one software. (A) Densitometric quantification of the western blotting gel data of TLR4 and TLR2 expression presented in Fig. 2A. (B) Densitometric quantification of the western blotting gel data presented in Fig. 3C. (C) Densitometric quantification of the western blotting gel data presented in Fig. 4B. These data are representative of three experiments and are shown as means ± SDEV (* p<0.05 by One-Way ANOVA with Tukey's post-hoc).



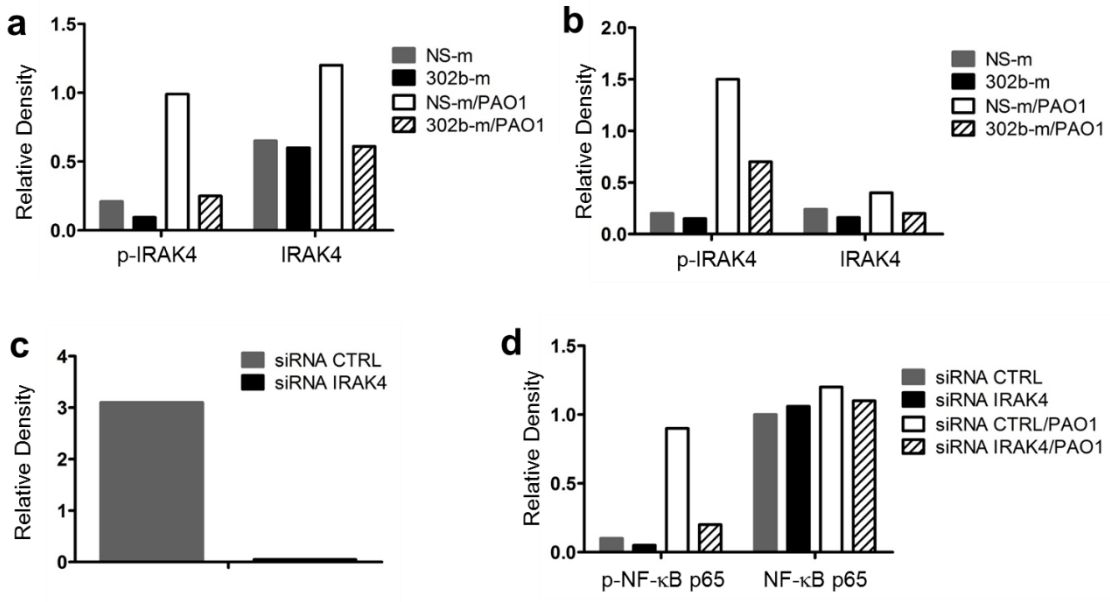
Supplementary Figure 4 | Time-dependent effects of miR-302b on bacterium–induced inflammatory cytokine gene expression. 24 hours after the transfection of MLE-12 cells with NS-m, 302b-m, NS-i and 302b-i, the cells were infected with PAO1 at MOI 10:1 for 1 h and polymyxin B (100 μg/ml) was added for another 1 h to kill bacteria outside of the cells. The cell samples were also collected at a different time points from 1 to 24 hours. The expression of IL-1β, IL-6, and TNF-α in MLE-12 cells were detected by real-time qPCR. Average values and SDEVs were calculated from triplicate samples.



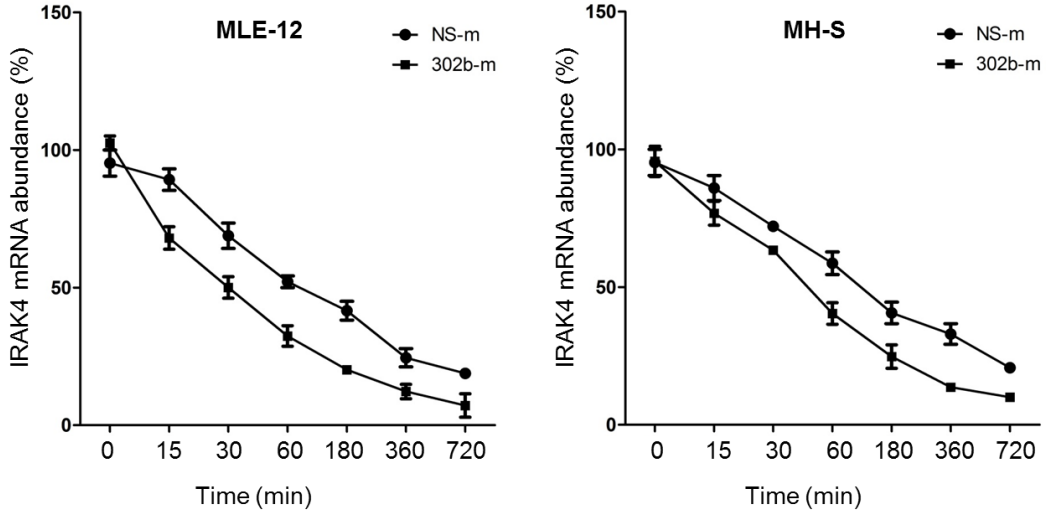
Supplementary Figure 5 | miR-302b does not suppress bacterium–induced anti-inflammatory gene expression. (**A**) Realtime qPCR analysis of IL-10 and IL-4 mRNA levels in MLE-12 cells transfected with NS-m, 302b-m, NS-i, or 302b-i, and infected with PAO1, PAK and Kp. (**B**) ELISA analysis of IL-10 and IL-4 protein levels in cell culture medium 16 h after PAO1, PAK and Kp treatment. MLE-12 cells were transfected as indicated in **A**. Average values and SDEVs were calculated from triplicate samples.



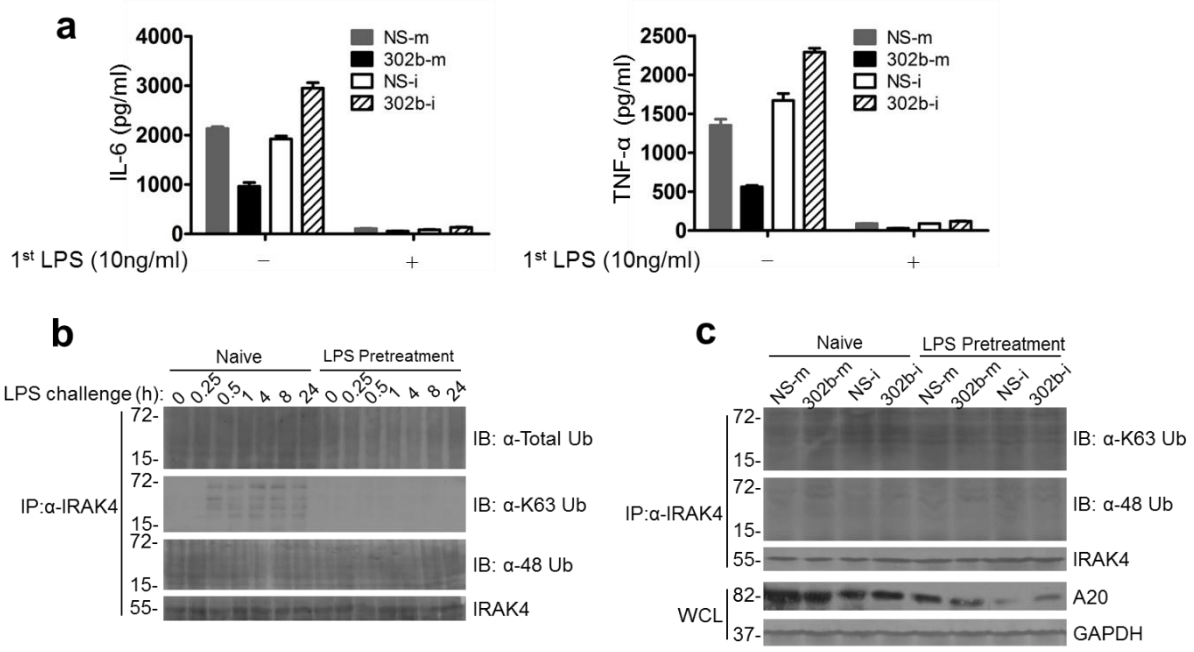
Supplementary Figure 6 | miR-302b inhibits bacterium–induced IL-6 and TNF-α expression in vivo. Mice were *i.v.* injected with vehicle, NS-m, or 302b-m (50 μg/mouse). 24 h later, mice were treated with or without 1×10^7 CFU/mouse of PAO1 for 12 h. Real-time qPCR analysis of IL-6 (**A**) and TNF-α (**B**) mRNA levels in indicated tissues was performed. These data are representative of three experiments and are shown as means ± SDEV.



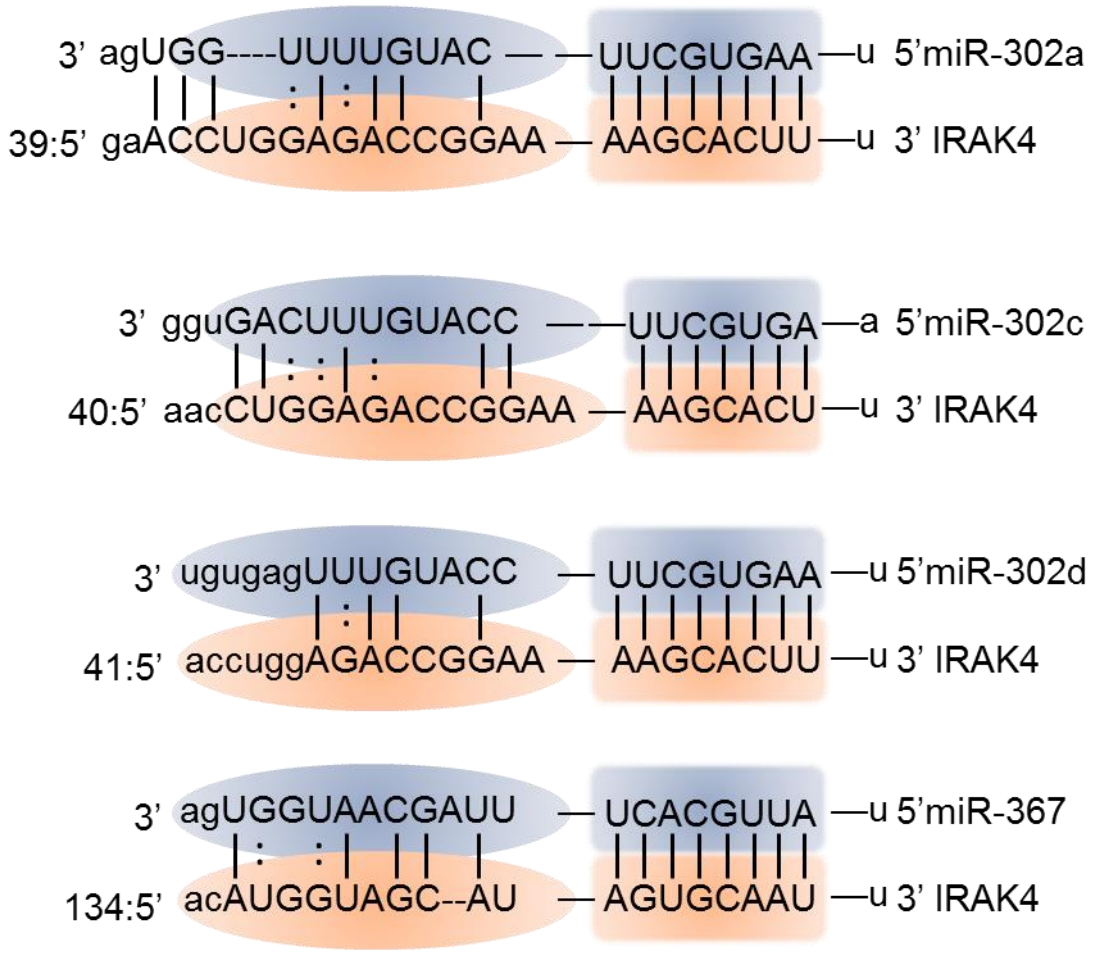
Supplementary Figure 7 | Densitometric quantification of the western blotting gel data presented in Fig. 7 (in text) using Quantity one software. (A) Densitometric quantification of the western blotting gel data presented in Fig. 7C. (B) Densitometric quantification of the western blotting gel data presented in Fig. 7F. (C) Densitometric quantification of the western blotting gel data presented in Fig.7G. (D) Densitometric quantification of the western blotting gel data presented in Fig.7H. These data are representative of three experiments and are shown as means.



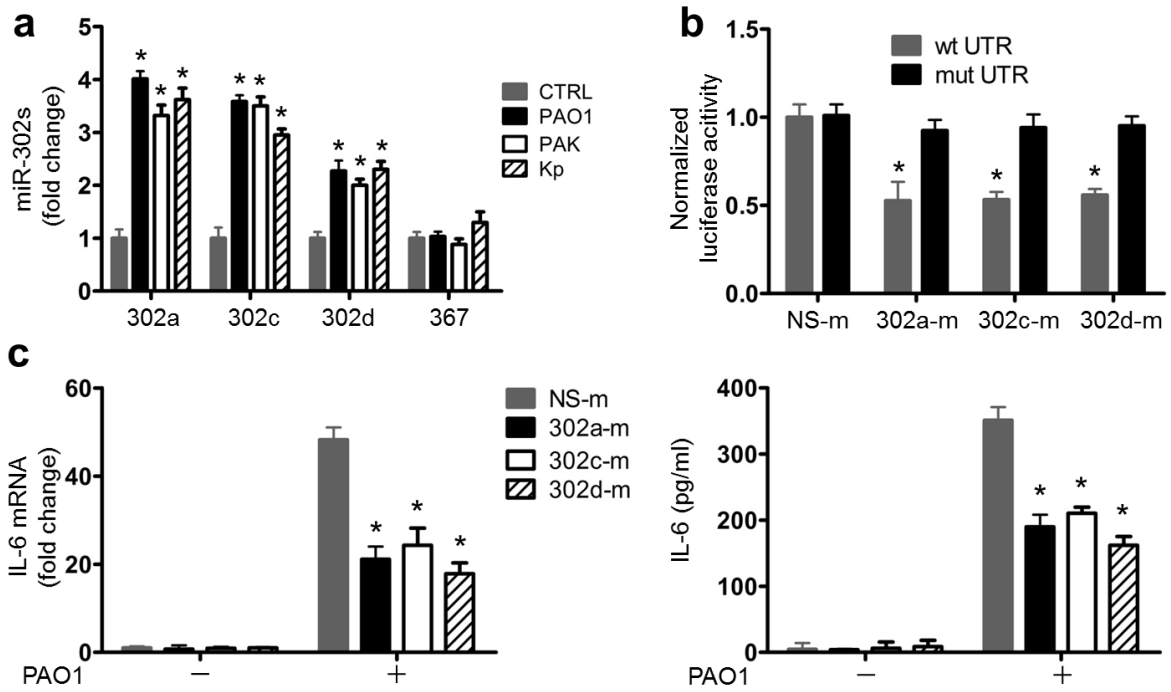
Supplementary Figure 8 | miR-320b regulates IRAK4 mRNA stability. 24 hours after transfection of MLE-12 and MH-S cells with NS-m and 302b-m, the cell samples were collected at different time points and IRAK4 mRNA abundance in MLE-12 and MH-S cells was detected by qPCR. These data are representative of three experiments and are shown as means ± SDEV.



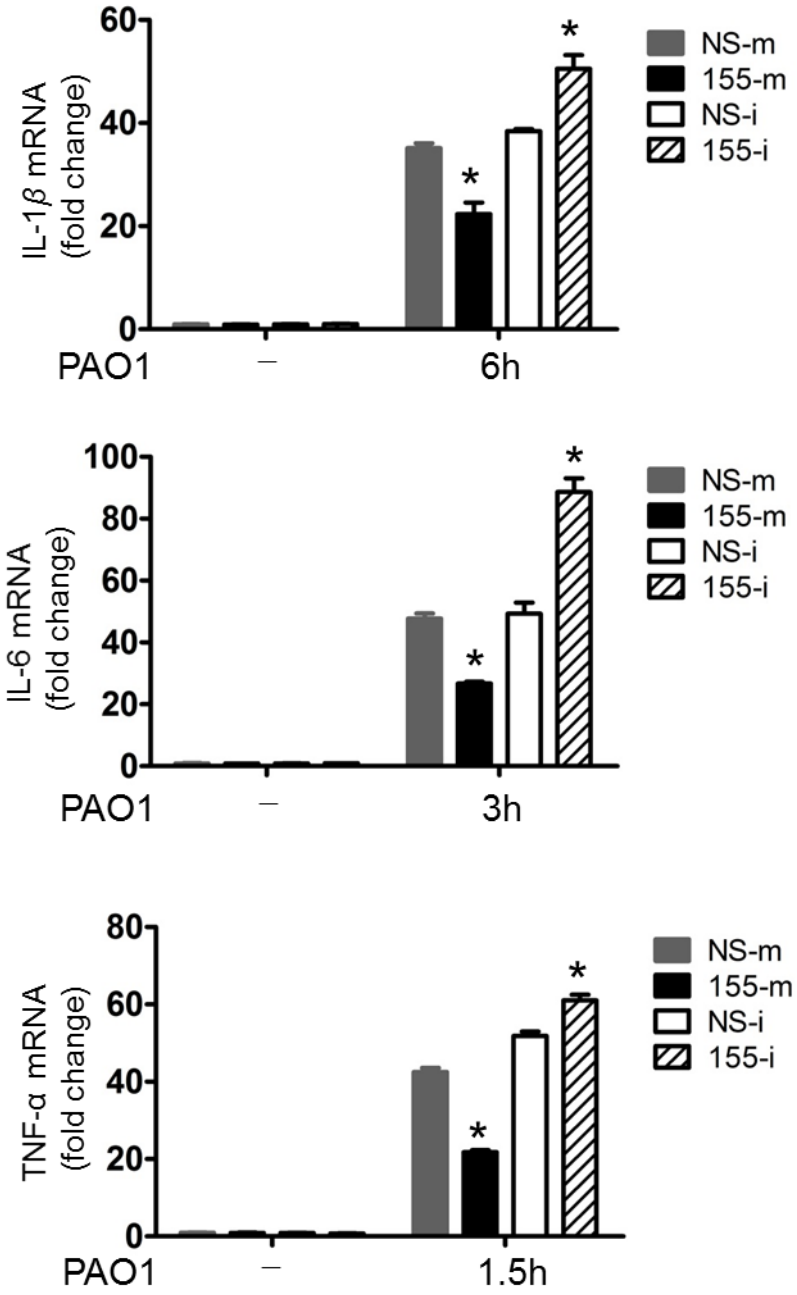
Supplementary Figure 9 | miR-320b does not regulate LPS tolerance. (**A**) 24 hours after transfection of MH-S cells with NS-m, 302b-m, NS-i, and 302b-i, the cells were pretreated for another 24 h with 10 ng/ml LPS, washed with PBS, and returned to fresh medium for 2 h. Naive and LPS-pretreated cells were stimulated with 100 ng/ml LPS for 2-6 h. TNFα (supernatants were collected at 2 h) and IL-6 (supernatants were collected at 6 h) levels in culture supernatants were measured by ELISA. Data are shown as means ± SDEVs and representative of three experiments (* p<0.05 by One-Way ANOVA with Tukey's post-hoc). (**B**) MH-S cells were pretreated for 24 h with 10 ng/ml LPS, washed with PBS, and returned to fresh medium for 2 h. Naive and LPS-pretreated cells were stimulated with 100 ng/ml LPS for 0-24 h. The cell samples were collected at different time points. IRAK4 was immunoprecipitated from cell lysates, followed by immunoblot analyses with Abs specific for total ubiquination (Ub), K63-linked Ub, and for K48-linked Ub chains. (**C**) The cell stimulated with 100 ng/ml LPS from (A) were collected at 4 h. IRAK4 was immunoprecipitated from cell lysates, followed by immunoblot analyses with Abs specific for K63-linked Ub and for K48-linked Ub chains. The expression of A20 in whole cell lysate (WCL) was also detected by western blot. GAPDH serves as the loading control. Data are representative of three experiments.



Supplementary Figure 10 | Other members of miR-302 family have the same binding site with miR-302b. IRAK4 3’UTRs contain one predicted binding site of miR-302a, miR-302c, miR-302d, and miR-367. The figure shows predicted duplex formations between IRAK4 3´UTR (bottom) and miR-302 family mature miRNA (top).



Supplementary Figure 11 | Other members of miR-302 family have the similar function to miR-302b. (**A**) Real-time qPCR analysis of miR-302a, miR-302c and miR-302d expression in MLE-12 cells after the infection of PAO1, PAK and Kp. (**B**) Normalized luciferase activity of a reporter containing the wild-type or point-mutated 3′ UTR reporter constructs (wt UTR or mut UTR) of IRAK4 in MLE-12 cells cotransfected with miRNA negative control (NS-m), miR-302a mimics (302a-m), miR-302c mimics (302c-m), miR-302d mimics (302d-m). (**C**) Realtime qPCR analysis of IL-6 mRNA levels (Left) and ELISA analysis of elaborated IL-6 protein levels (Right) in MLE-12 cells transfected with miRNA NS-m, 302a-m, 302c-m, 302d-m and infected with PAO1. Average values and SDEVs were calculated from triplicate samples (* $p<0.05$ by One-Way ANOVA with Tukey's post-hoc).



Supplementary Figure 12 | miR-155 has the similar function to miR-302b. 24 hours after transfection of MH-S cells with NS-m, 302b-m, NS-i, and 302b-i, cells were infected with PAO1 at MOI 10:1 for 1 h and polymyxin B (100 μg/ml) was added for another 1 h to kill bacteria outside of the cells. The cell samples were also collected at different time points from 1.5 to 6 hours. The expression of IL-1β, IL-6, and TNF-α in MH-S cells was detected by real-time qPCR. Average values and SDEVs were calculated from triplicate samples (* $p<0.05$ by One-Way ANOVA with Tukey's post-hoc).

Supplementary Table 1 | Up-regulated miRNAs upon bacterial infection.

Mature ID	Fold Change (up)
mmu-miR-182-5p	3.8187
mmu-miR-200c-3p	3.9795
mmu-miR-26a-5p	2.0946
mmu-miR-294-3p	2.1229
mmu-miR-302b-3p	16.2438
mmu-miR-495-3p	5.561
mmu-miR-669k-3p	3.3576
mmu-miR-155-5p	1.9516

Supplementary Table 2 | Primers of miRNAs and other genes.

ID	Primer Sequences (5'-3')
miR-302b-3p Sense	GCGGCGTAAGTGCTTCCATGTTTTA
miR-302a-3p Sense	GCGGCGTAAGTGCTTCCATGTTTTG
miR-302c-3p Sense	GCGGCGTAAGTGCTTCCATGTTTCA
miR-302d-3p Sense	GCGGCGTAAGTGCTTCCATGTTTGA
snoRNA202 Sense	GCCTTTTGAACCCTTTTCCATCTG
IRAK4 Sense	GTGAACAACACCATCGTGGC
IRAK4 Antisense	GAGCAACCTTGCACCTTGTG
IL-1β Sense	GTCAACGTGTGGGGGATGAA
IL-1β Antisense	AAGCAATGTGCTGGTGCTTC
IL-6 Sense	CCCCAATTTCCAATGCTCTCC
IL-6 Antisense	CGCACTAGGTTTGCCGAGTA
TNF-α Sense	GGCAGGTTCTGTCCCTTTCA
TNF-α Antisense	CATCTTTTGGGGGAGTGCCT

Supplementary Table 3 | Primers of luciferase reporter construct containing IRAK4 3′ UTR.

ID	Primer Sequences (5'-3')
pGL3-Luc IRAK4 Sense	CTCGTCTAGATTTACTTCTCGAGAACCTGGAGACCGG
pGL3-Luc IRAK4 Antisense	ATAATCGGCCGGCCTTCCTTACAGGAACCTAGGT
pGL3-Luc IRAK4 Mutant Sense	AACCTGGAGACCGGAAGTAATATTTGACACTGAGCTGCGTCACCTA