miR-718 represses pro-inflammatory cytokine production through targeting PTEN

Parisa Kalantari, Omid F. Harandi, Sarika Agarwal, Florentina Rus, Evelyn A. Kurt-Jones, Katherine A. Fitzgerald, Daniel R. Caffrey, Douglas T. Golenbock

SUPPLEMENTARY DATA

Supplementary TABLE 1: List of miRNAs hosted by innate immune protein-coding genes. Ensembl API, version 52 was used to retrieve gene ontology terms for all protein coding genes that hosted a miRNA.

Supplementary FIGURE 1: miR-718 is induced in iBMDMs stimulated with LOS but not poly (I:C). (A) Wild type iBMDMs were unstimulated or stimulated with 100 ng/ml of LOS or 5μg/ml poly (I:C) for 2h. (B) Wild type iBMDMs were stimulated with 100 ng/ml of LPS for 2h. RNA was extracted and miR-718 or miR-146 expression was quantitated by TaqMan qPCR. Levels of microRNA expression are shown relative to levels of snoRNA-202. Data shown represent the mean ± S.D. of triplicate determinations and are representative of three independent experiments with similar results.

Supplementary FIGURE 2: Expression of miR-718 is inversely correlated with TNFα and IL-1β. (A) Con Inh, anti-miR-718 and IRAK1/miR-718-dKO macrophages were unstimulated (Medium) or stimulated with LPS (100ng/ml and 200ng/ml) for 2h. ELISA was performed to determine TNF-α production. (B) Con Inh and anti-miR-718 macrophages were stimulated with various concentrations of LPS and TNF-α production was measured by ELISA. (C) Con Inh and anti-miR-718 macrophages were stimulated with 100ng/ml of LPS for 2h and either untreated or treated with 10 μM of Nigericin for 1 h. ELISA was performed to determine IL-1β production. (D) Con Inh and anti-miR-718 macrophages were either uninfected (Medium), infected with various MOIs of *N. gonorrhoeae*, or stimulated with LPS. ELISA was performed to determine TNF-α production. Data shown represent the mean ± S.D. of triplicate determinations and are representative of three independent experiments with similar results.

Supplementary FIGURE 3: In the absence of miR-718, NF-κB activity is increased. (A) Con Inh and anti-miR-718 macrophages were transiently transfected with 1 μg of the NF-κB-Luc construct and 1 μg of the miR-718 construct or empty vector using lipofectamine 2000 and then stimulated with various concentrations of LPS or just stimulated with LPS for 2h and assayed for luciferase activity. After 48 h, 293T cells were lysed and the normalized firefly luciferase activity (firefly luciferase activity/renilla luciferase activity) was calculated. Data shown represent the mean ± S.D. of triplicate determinations and are representative of three independent experiments with similar results.

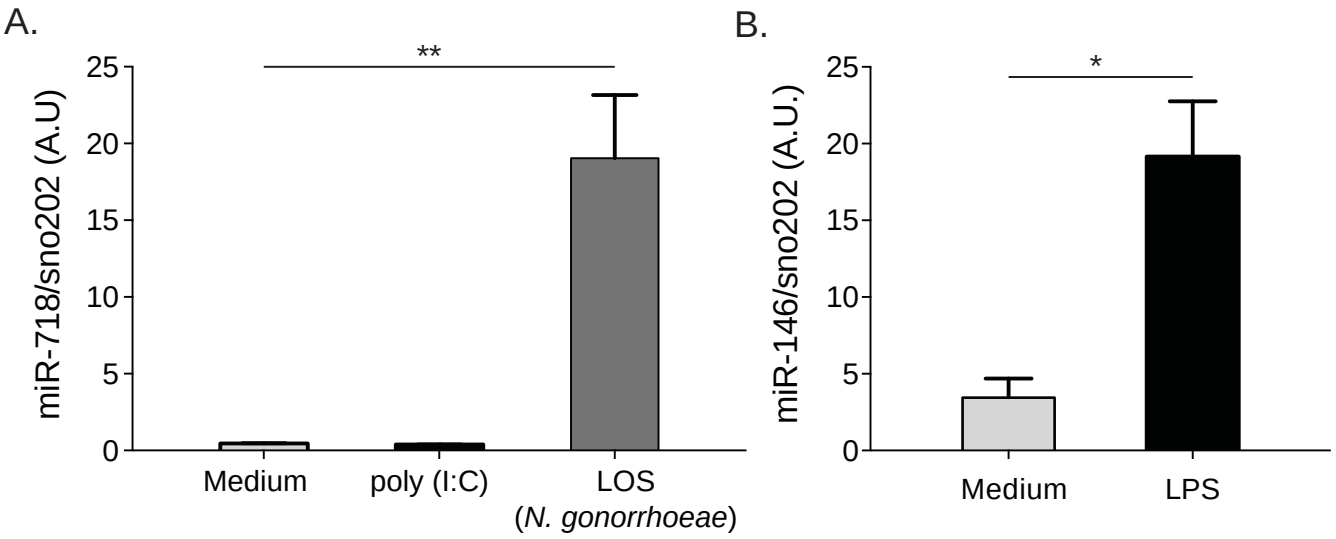
Supplementary FIGURE 4: miR-718 directly represses PTEN expression in macrophages. (A) The PTEN-3'UTR-Luc construct was co-transfected with various concentrations (10nM, 50nM and 100nM) of the miR-718 construct or the miRNA negative Control (Con miR) into 293T cells. After 48 h, 293T cells were lysed and the normalized firefly luciferase activity (firefly luciferase activity/renilla luciferase activity) was calculated. (B) Con Inh and anti-miR-718 macrophages stimulated with 100ng/ml LPS for 2h were fixed, permeabilized, and stained with PTEN antibody and DAPI (nucleus) and subjected to confocal microscopy. Scale bars, all 20 μm. Images are representative of at least ten fields of view and three independent experiments. (C) Con Inh and anti-miR-718 macrophages stimulated with 100ng/ml LPS for 2h were fixed, permeabilized and stained with phosphorylated Akt (P-Akt) antibody and DAPI (nucleus) and subjected to confocal microscopy. Scale bars, all 20 μm. Images are representative of at least ten fields of view and three independent experiments. (D) Immortalized Con miR, miR-718, IRAK1-KO and TLR4-KO macrophages were either untreated (Medium) or pretreated with 5μM of API-1 (Akt inhibitor) and then stimulated with LPS for 2h or unstimulated. TNF-α production was measured by

ELISA. (E) Immortalized Con miR, miR-718, IRAK1-KO and TLR4-KO macrophages were either untreated (Medium) or pretreated with 5μM of MK-2006 (Akt inhibitor) and then stimulated with LPS for 2h or unstimulated. TNF-α production was measured by ELISA. (F) Immortalized Con Inh and anti-miR-718 macrophages were either untreated (Medium) or pretreated with 10μM of Akt inhibitor IV and then stimulated with LPS for 2h or unstimulated. TNF-α production was measured by ELISA. Data shown represent the mean ± S.D. of triplicate determinations and are representative of three independent experiments with similar results.

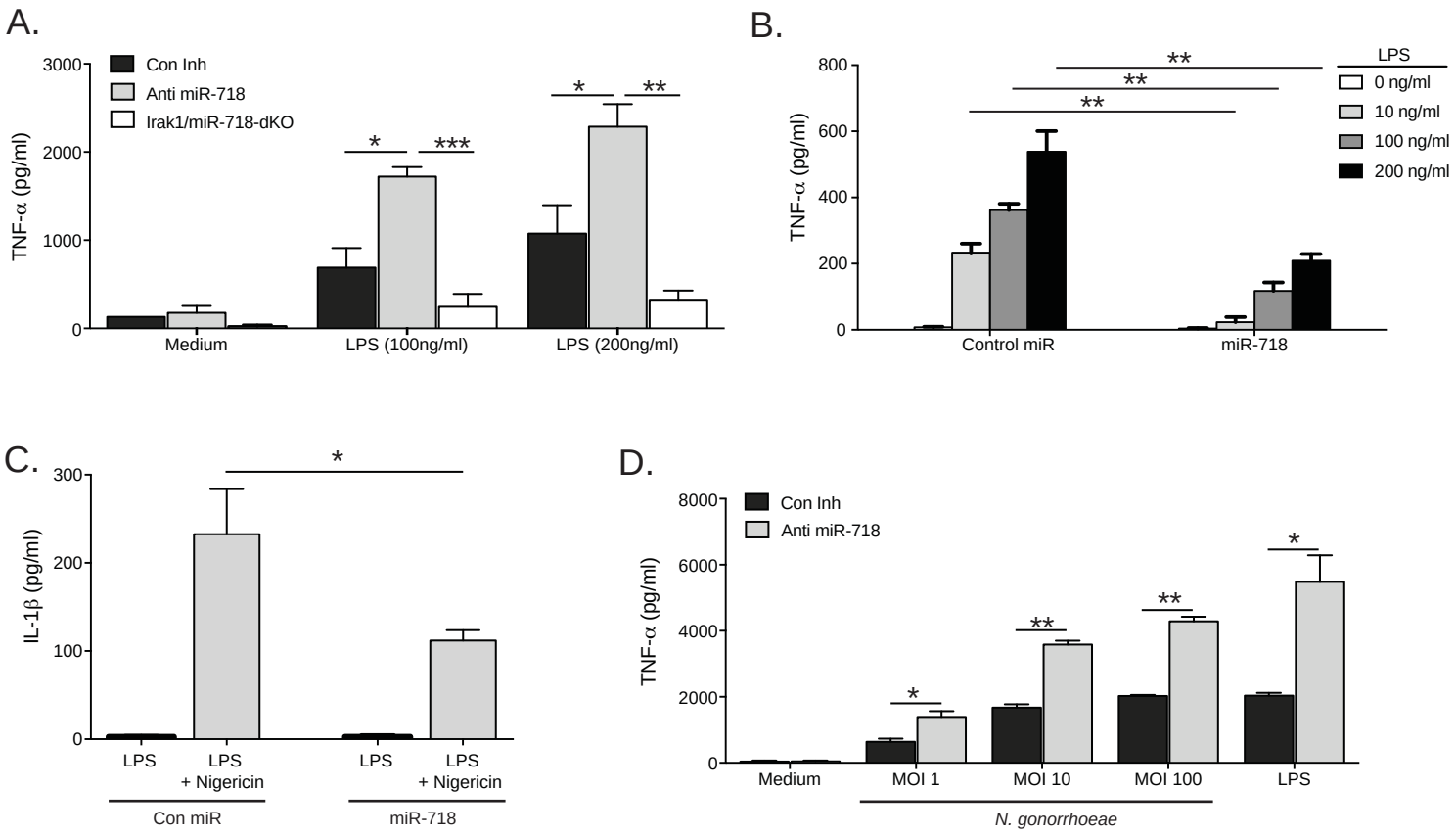
Supplementary FIGURE 5: miR-718 suppresses TLR4 expression via Akt induced production of let-7e. (A) Immortalized Con miR, miR-718 and TLR4-KO macrophages were either untreated or pretreated with 5μM of Akt Inhibitor MK-2206 and then stimulated with LPS for 2h. RNA was extracted and TLR4 expression was quantitated by SYBR Green qPCR. Levels of TLR4 are shown relative to levels of β-actin. (B) Immortalized Con miR and miR-718 macrophages were either untreated or pretreated with 5μM of Akt inhibitor MK-2206 and then stimulated with LPS for 2h. RNA was extracted and let-7e expression was quantitated by Taqman qPCR. Levels of let-7e are shown relative to levels of sno-202. (C) Immortalized Con miR and miR-718 macrophages were transfected with 50nM let-7e or scrambled (SCR)-miRNA using Lipofectamine 2000. Cells were then stimulated with LPS for 2h or unstimulated. TNF-α production was measured by ELISA. Data shown represent the mean ± S.D. of triplicate determinations and are representative of three independent experiments with similar results.

Supplementary FIGURE 6: miR-718 regulates the induction of LPS tolerance and *N. gonorrhoeae* infection (A) Con miR and miR-718 or (B) Con Inh and Anti-miR-718 macrophages were primed with 10 ng/ml LPS continuously for 18 h (tolerized), washed twice with PBS and stimulated with 0, 10, 100, or 500 ng/ml LPS for 5 h. TNF-α production was measured by ELISA. (C) Con Inh and Anti-miR-718 or (D) Con miR and miR-718 macrophages were infected with *N. gonorrhoeae* (strain FA1090) for 1h, MOI 10. After 1 h, macrophages were washed three times to remove the extracellular bacteria and then the cells were lysed using 0.5% saponin. Dilutions of cell lysates were plated on gonococcal medium based agar and after 24 h bacterial CFUs were counted. Data shown represent the mean ± S.D. of triplicate determinations and are representative of three independent experiments with similar results.

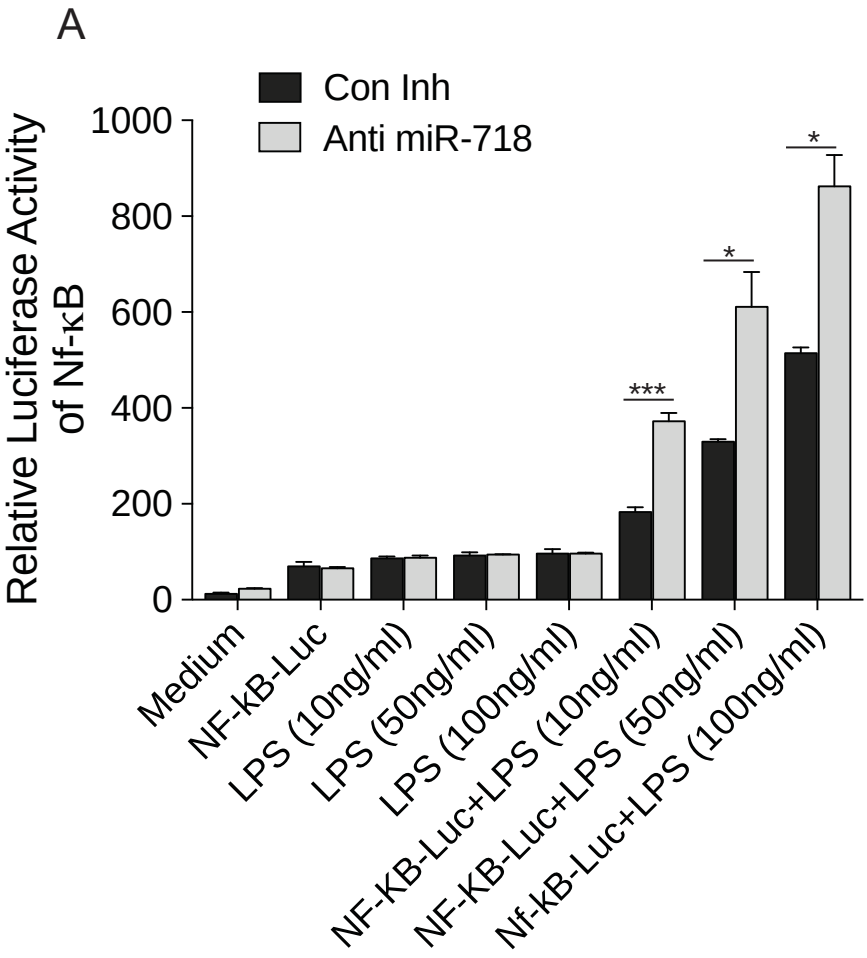
S. Figure 1


A.
**
miR-718/sno202 (A.U)
25
20
15
10
5
0
Medium
poly (I:C)
LOS
(N. gonorrhoeae)
B.
*
miR-146/sno202 (A.U.)
25
20
15
10
5
0
Medium
LPS

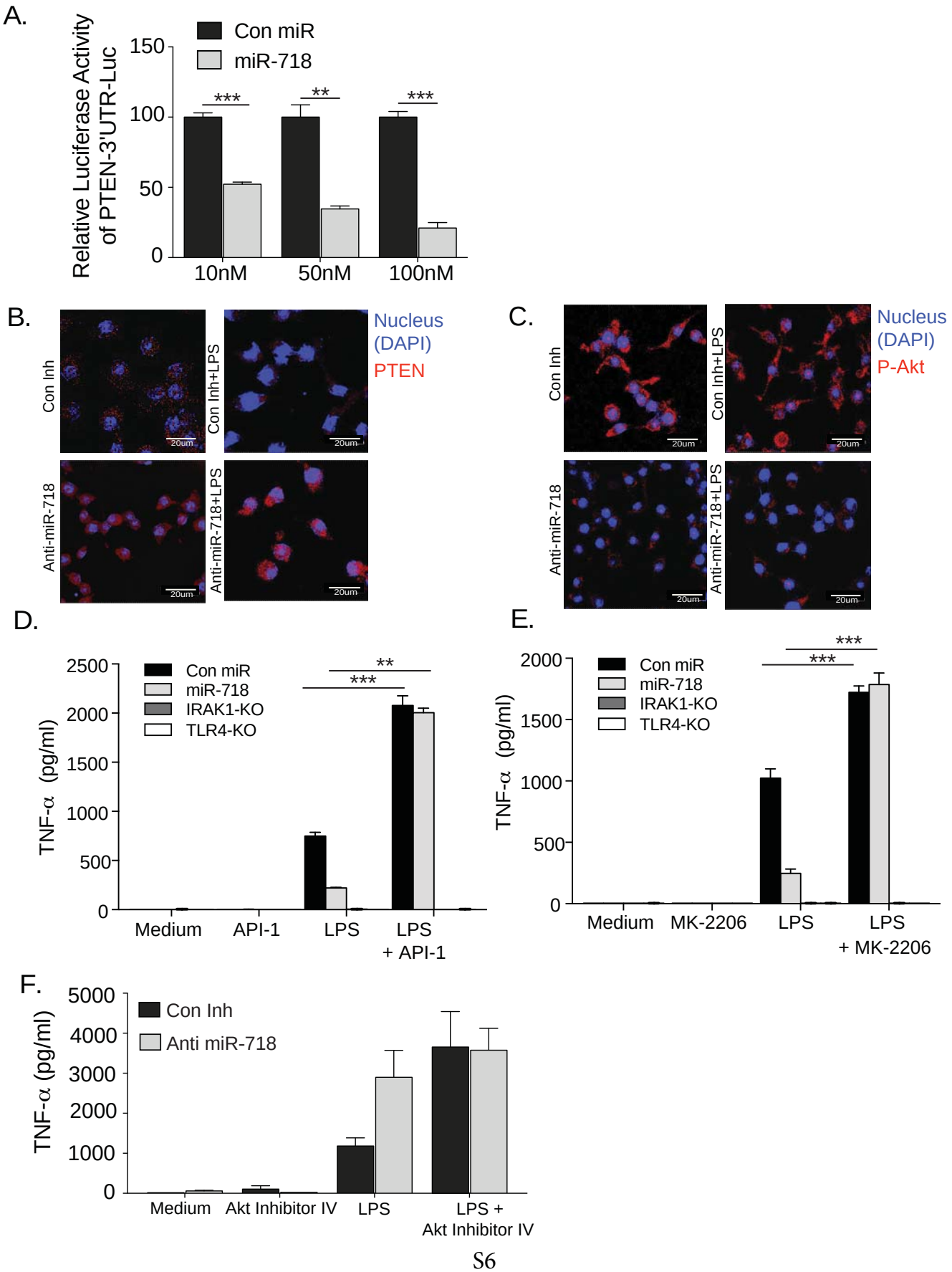
S. Figure 2


A.
Con Inh
Anti miR-718
Irak1/miR-718-dKO
TNF-α (pg/ml)
3000
2000
1000
0
*
***
*
**
Medium
LPS (100ng/ml)
LPS (200ng/ml)
B.
LPS
0 ng/ml
10 ng/ml
100 ng/ml
200 ng/ml
TNF-α (pg/ml)
800
600
400
200
0
**
**
**
Control miR
miR-718
C.
IL-1β (pg/ml)
300
200
100
0
*
LPS
LPS + Nigericin
LPS
LPS + Nigericin
Con miR
miR-718
D.
Con Inh
Anti miR-718
TNF-α (pg/ml)
8000
6000
4000
2000
0
*
**
**
*
Medium
MOI 1
MOI 10
MOI 100
LPS
N. gonorrhoeae

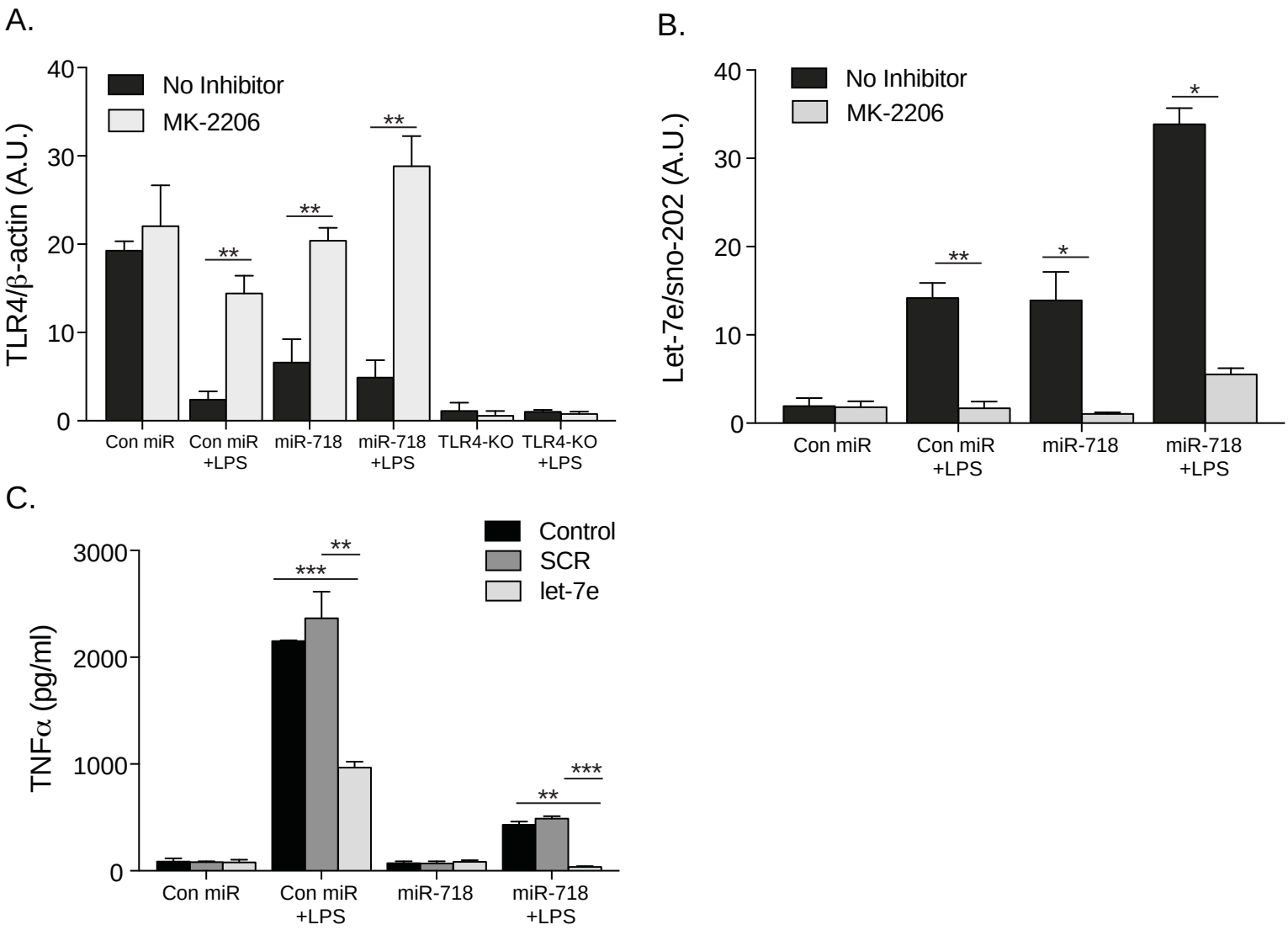
S. Figure 3


A
Con Inh
Anti miR-718
Relative Luciferase Activity of Nf-κB
1000
800
600
400
200
0
***
*
*
Medium
NF-kB-Luc
LPS (10ng/ml)
LPS (50ng/ml)
LPS (100ng/ml)
NF-KB-Luc+LPS (10ng/ml)
NF-KB-Luc+LPS (50ng/ml)
Nf-kB-Luc+LPS (100ng/ml)

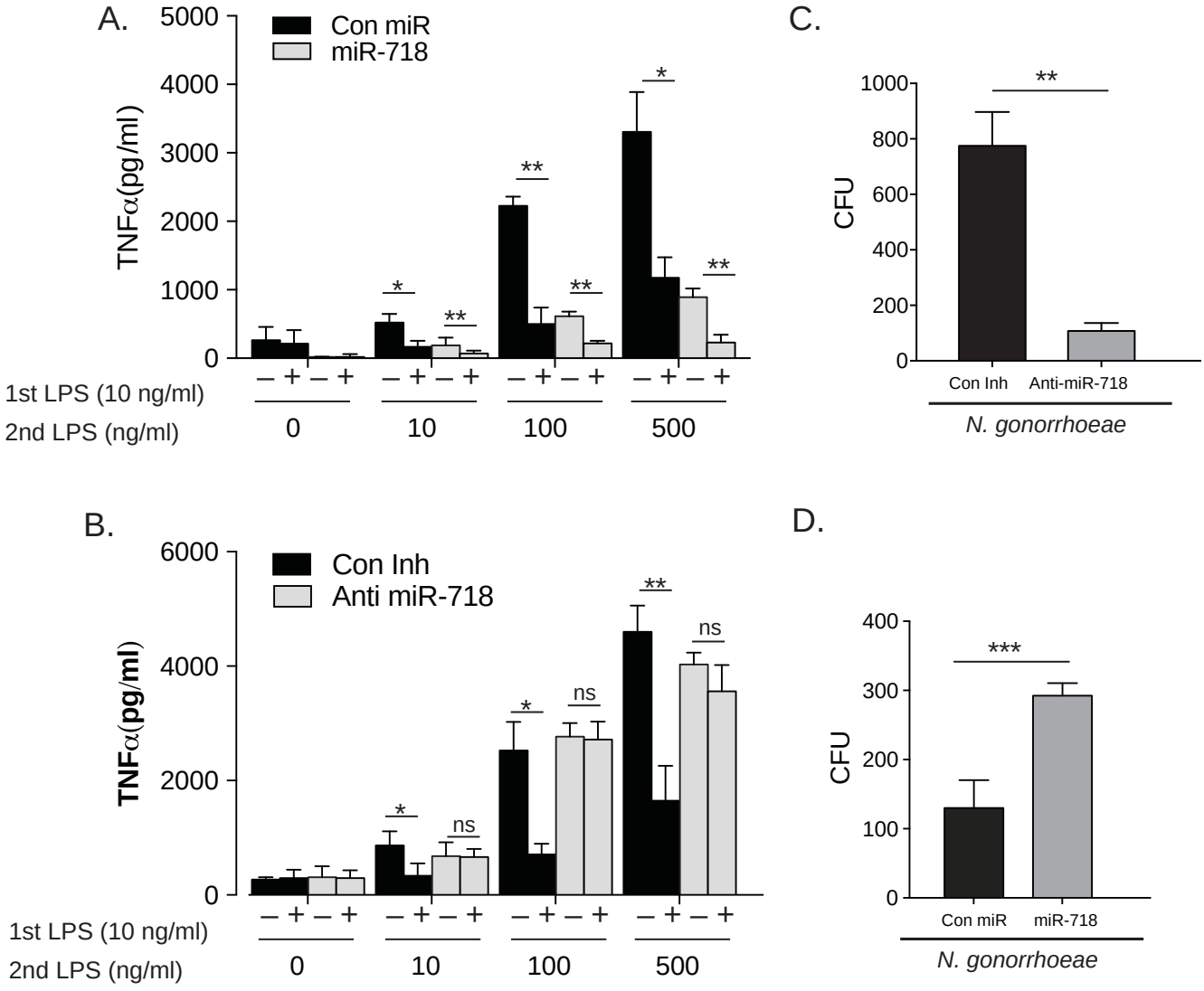
S. Figure 4


A.
Relative Luciferase Activity of PTEN-3'UTR-Luc
Con miR
miR-718
150
100
50
0
***
**
***
10nM
50nM
100nM
B.
Con Inh
Con Inh+LPS
Anti-miR-718
Anti-miR-718+LPS
Nucleus (DAPI)
PTEN
20um
C.
Con Inh
Con Inh+LPS
Anti-miR-718
Anti-miR-718+LPS
Nucleus (DAPI)
P-Akt
20um
D.
TNF-α (pg/ml)
Con miR
miR-718
IRAK1-KO
TLR4-KO
2500
2000
1500
1000
500
0
**
***
Medium
API-1
LPS
LPS + API-1
E.
TNF-α (pg/ml)
Con miR
miR-718
IRAK1-KO
TLR4-KO
2000
1500
1000
500
0
***
***
Medium
MK-2206
LPS
LPS + MK-2206
F.
TNF-α (pg/ml)
Con Inh
Anti miR-718
5000
4000
3000
2000
1000
0
Medium
Akt Inhibitor IV
LPS
LPS + Akt Inhibitor IV

S. Figure 5


A.
TLR4/β-actin (A.U.)
No Inhibitor
MK-2206
Con miR
Con miR +LPS
miR-718
miR-718 +LPS
TLR4-KO
TLR4-KO +LPS
B.
Let-7e/sno-202 (A.U.)
C.
TNFα (pg/ml)
Control
SCR
let-7e

S. Figure 6


A.
Con miR
miR-718
TNFα(pg/ml)
5000
4000
3000
2000
1000
0
1st LPS (10 ng/ml)
2nd LPS (ng/ml)
0
10
100
500
C.
CFU
1000
800
600
400
200
0
Con Inh
Anti-miR-718
N. gonorrhoeae
B.
Con Inh
Anti miR-718
TNFα(pg/ml)
6000
4000
2000
0
ns
1st LPS (10 ng/ml)
2nd LPS (ng/ml)
0
10
100
500
D.
CFU
400
300
200
100
0
Con miR
miR-718
N. gonorrhoeae