Three paralogous clusters of miR-17~92 family microRNAs restrain IL-12-mediated immune defense

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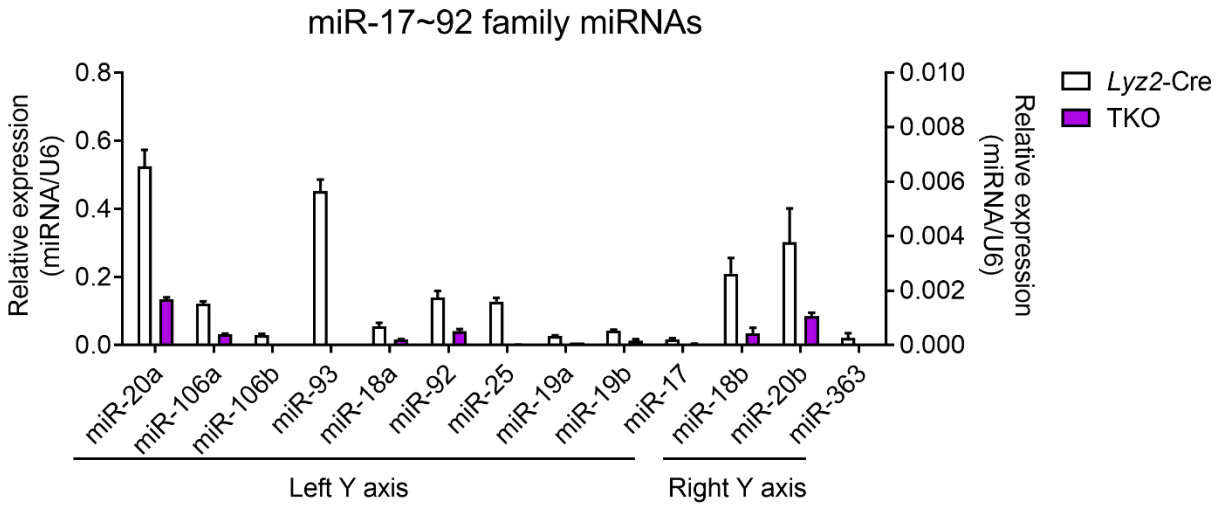
Supplementary Figure 1

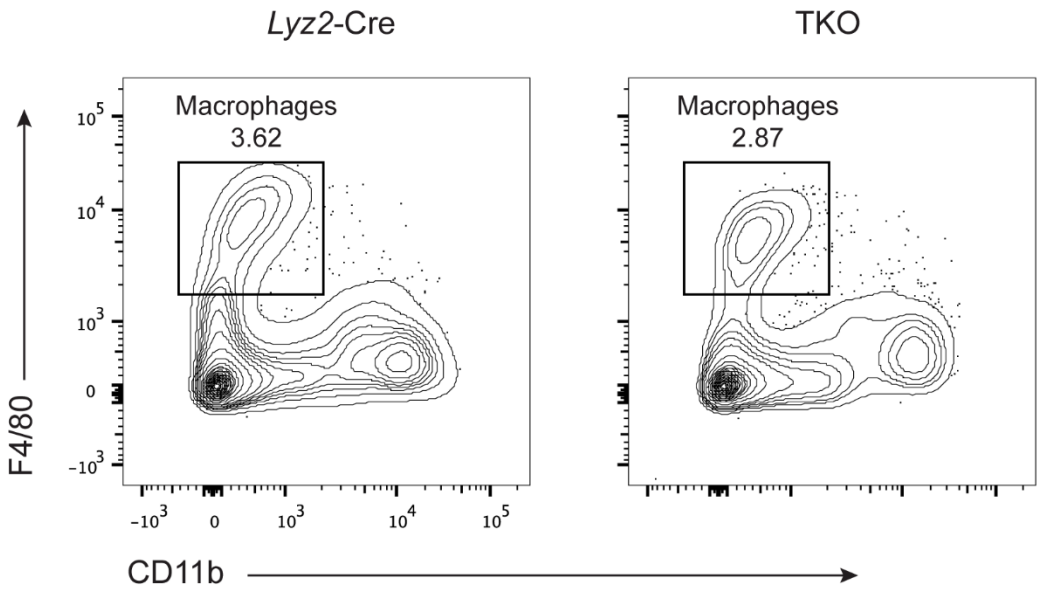


Supplementary Figure 1

The miR-17~92 family miRNAs are efficiently deleted in TKO BMDMs.

qPCR analysis of various mature miRNAs (horizontal axes) in *Lyz2*-Cre and *miR-106a~363*$^{-/-}$ *miR-106b~25*$^{-/-}$ *miR-17~92*$^{flox/flox}$ *Lyz2*-Cre (TKO) BMDMs; results are presented as relative expression normalized to the control small RNA U6. Data are representative of two independent experiments. Data are presented as mean + SD.

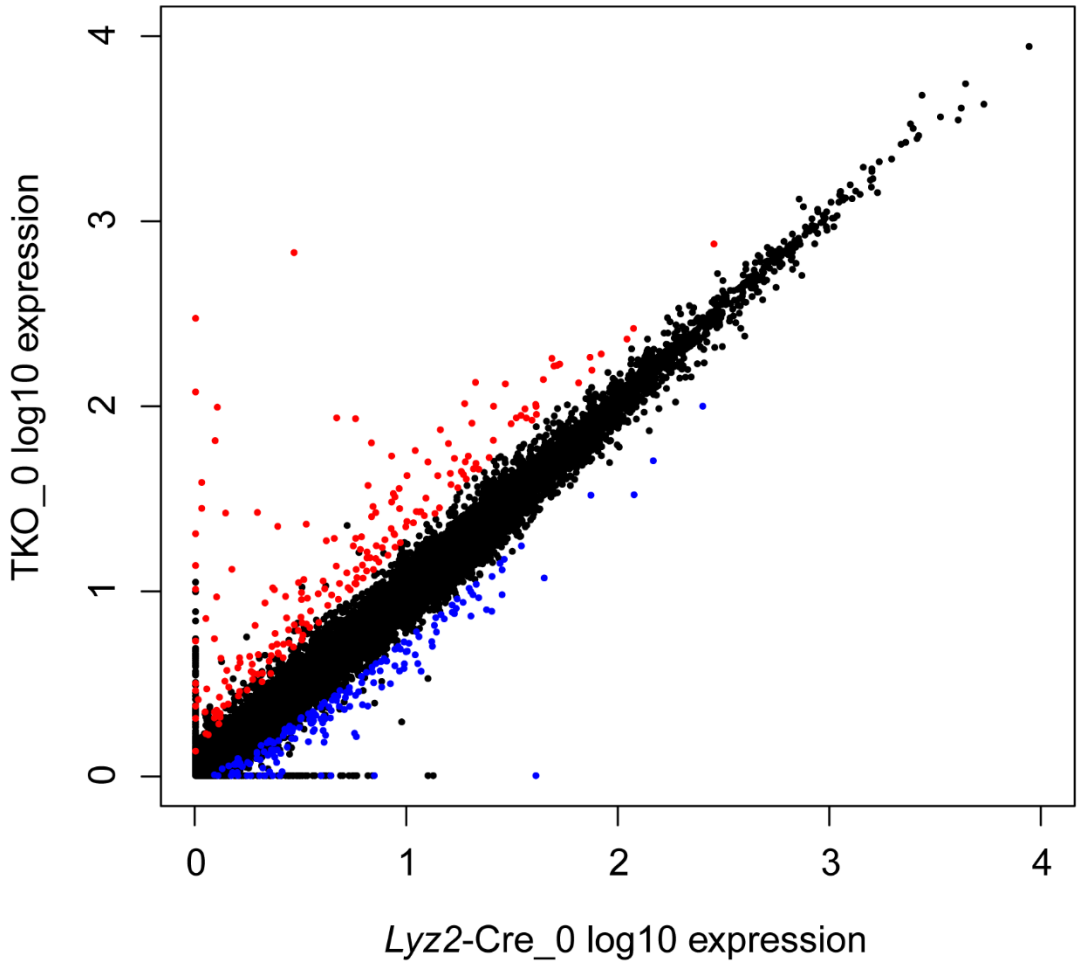
Supplementary Figure 2



Supplementary Figure 2

The miR-17~92 family miRNA deficient mice exhibit normal macrophage populations. FACS analysis of macrophage populations in the spleen of *Lyz2*-Cre and TKO mice under homeostatic conditions. Results of one representative experiment out of two performed are shown.

Supplementary Figure 3

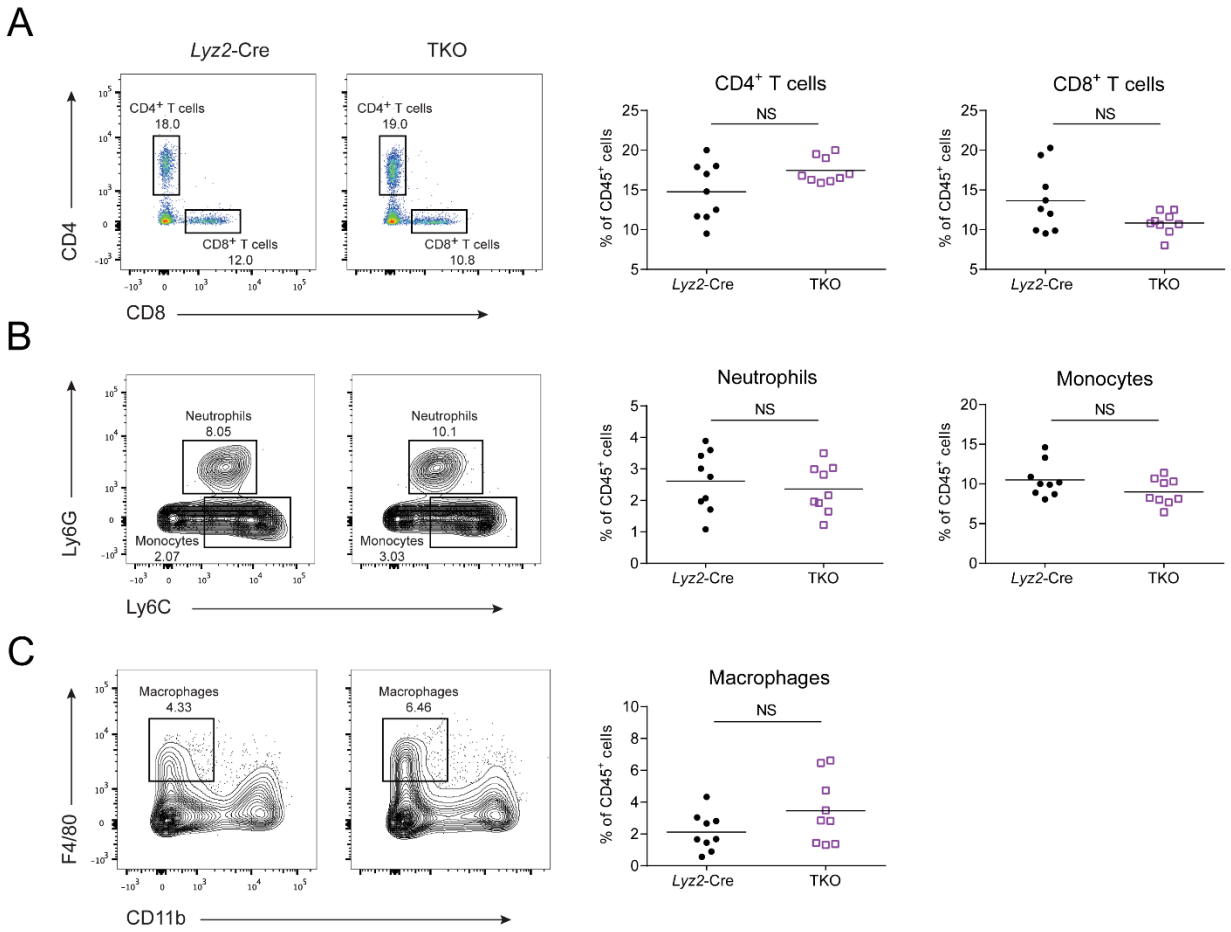


Supplementary Figure 3

miR-17~92 family miRNAs do not regulate *Il12a* and *Il12b* expression under homeostatic condition in macrophages.

RNA-seq analysis showing RNA expression in TKO BMDMs versus those in *Lyz2*-Cre cells without stimulation. RNAs up-regulated in TKO BMDMs were colored red, whereas RNAs down-regulated were colored blue.

Supplementary Figure 4



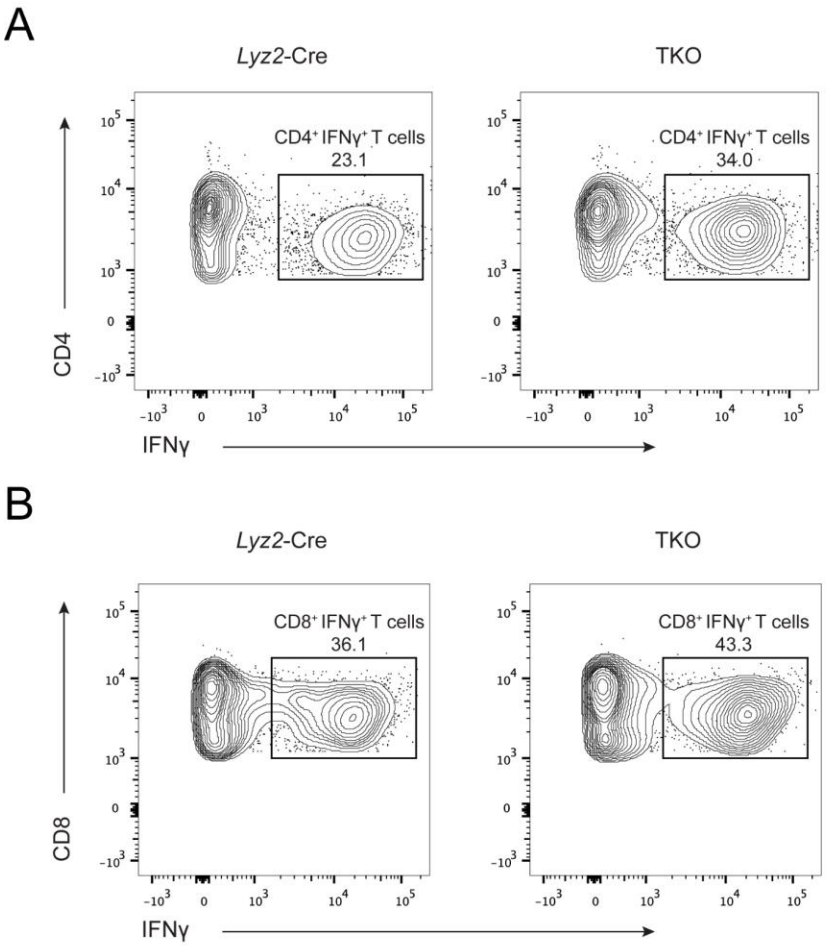
Supplementary Figure 4

miR-17~92 family miRNA deficiency does not alter cell populations in *Listeria monocytogenes* infected mice.

FACS analysis of $CD4^+$ and $CD8^+$ T cells (A), monocytes and neutrophils (B), and macrophages (C) in the spleen of *Lyz2*-Cre and TKO mice infected with 5×10^4 *L. monocytogenes* for 6 days. FACS plots from one representative experiment is shown on the left panels and percentages of each cell population from 9 mice are quantified as mean values in the right panels. Data are pooled from two independent experiments (n = 9 mice per group).

Statistical significance was calculated using Unpaired Student's *t*-test (NS, not significant, $P > 0.05$).

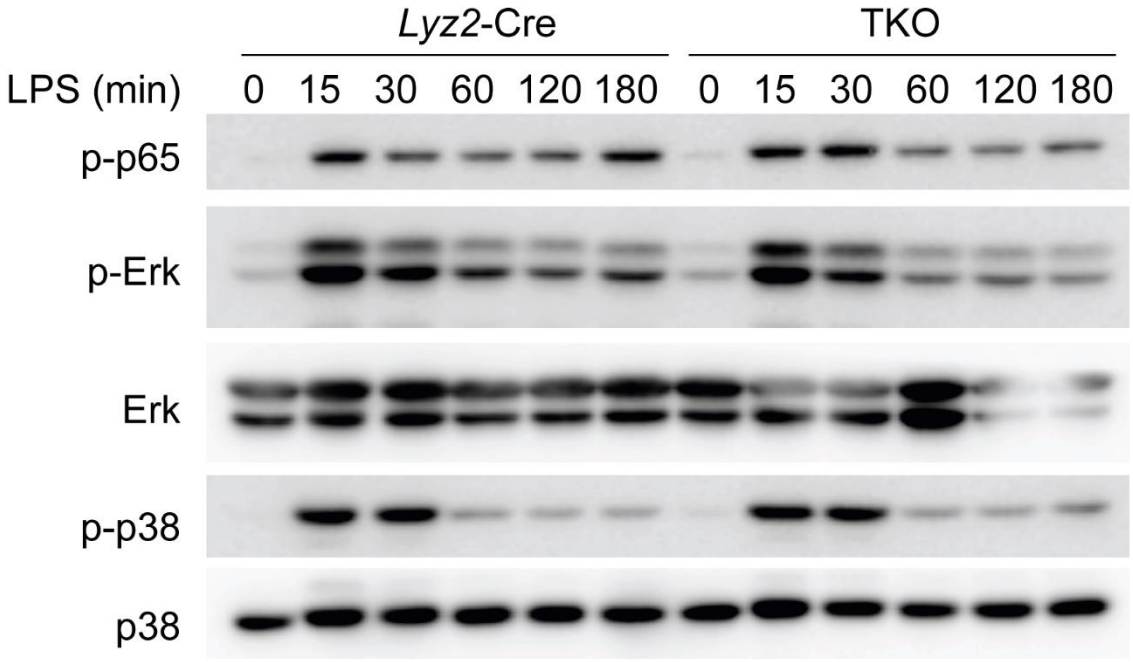
Supplementary Figure 5



Supplementary Figure 5

miR-17~92 family miRNA deficiency promotes IFNγ production in CD4$^+$ and CD8$^+$ T cells. FACS analysis of IFNγ producing CD4$^+$ (A) and CD8$^+$ (B) T cells in the spleen of *Lyz2*-Cre and TKO mice infected with 5×10^4 *L. monocytogenes* for 8 days. Results of one representative experiment out of two performed are shown.

Supplementary Figure 6

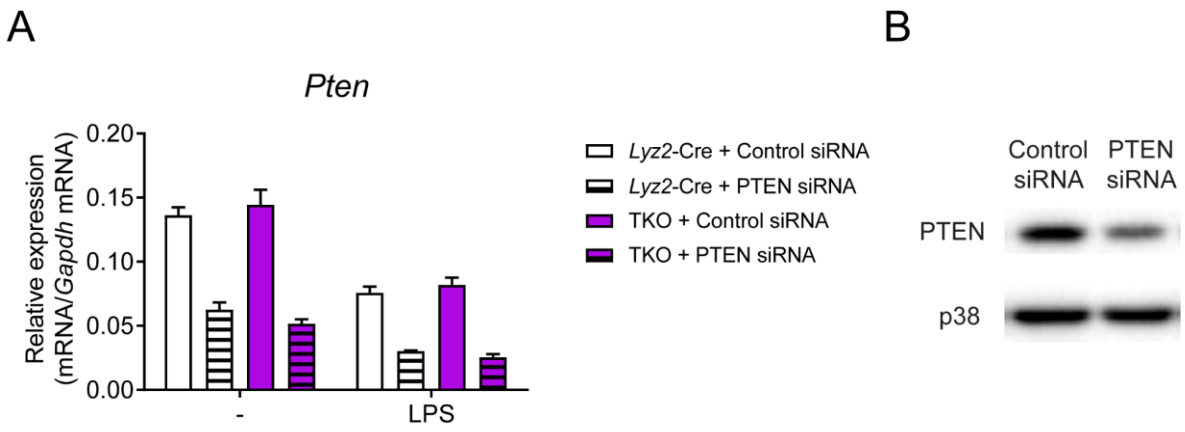


Supplementary Figure 6

miR-17~92 family miRNA deficiency does not alter activation of canonical NF-κB and MAPK signaling pathways.

Immunoblotting analysis of phosphorylated p65, phosphorylated and total Erk, phosphorylated and total p38 in whole-cell lysates of *Lyz2*-Cre and TKO BMDMs treated for the indicated periods with LPS. Data are representative of three independent experiments.

Supplementary Figure 7



Supplementary Figure 7

PTEN expression is efficiently knocked down by RNA interference.

(A) *Lyz2*-Cre and TKO BMDMs were transfected with control non-targeting or PTEN specific siRNA. 72 h post transfection, cells were stimulated with LPS for 3 h and *Pten* mRNA levels were assessed by qPCR. Results are presented as relative expression normalized to *Gapdh* mRNA.

(B) Immunoblotting analysis of PTEN and p38 (loading control) in whole-cell lysates of TKO BMDMs transfected with siRNAs and stimulated with LPS as in (A). Data are representative of three independent experiments.

Data are presented as mean + SD.

Supplementary Table 1. Primers used in this study.

Gene	Forward Primer (5'>3')	Reverse Primer (5'>3')
	Primer sequences for regular qPCR	
Il12a	ACGGGACCAAACCAGCACATT	AAGGCACAGGGTCATCATCAAAGA
Il12b	AGCACTCCCCATTCCTACTTCTCC	CACCCCTCCTCTGTCTCCTTCAT
Pten	CATGACAGCCATCATCAAAGA	TCTGCAGGAAATCCCATAGC
Gapdh	ATCAAGAAGGTGGTGAAGCA	AGACAACCTGGTCCTCAGTGT
	Primer sequences for small RNA qPCR	
miR-17	CAAAGUGCUUACAGUGCAGGUAGU	
miR-20a	UAAAGUGCUUAUAGUGCAGGUAG	
miR-20b	CAAAGUGCUCAUAGUGCAGGUA	
miR-106a	CAAAGUGCUAACAGUGCAGGUA	
miR-106b	UAAAGUGCUGACAGUGCAGAU	
miR-93	CAAAGUGCUGUUCGUGCAGGUAG	
miR-18a	UAAGGUGCAUCUAGUGCAGAUA	
miR-18b	UAAGGUGCAUCUAGUGCAGUUA	
miR-19a	UGUGCAAAUCUAUGCAAAACUGA	
miR-19b	UGUGCAAAUCCAUGCAAAACUGA	
miR-92	UAUUGCACUUGUCCCGGCCUG	
miR-25	CAUUGCACUUGUCUCGGUCUGA	
miR-363	AAUUGCACGGUAUCCAUCUGUAA	
U6	TGGCCCCTGCGCAAGGATG	
Universal reverse primer		GCGAGCACAGAATTAATACGACTCAC