Online data supplement belonging to: **IFN-γ Induction by Neutrophil-derived IL-17A Homodimer Augments Pulmonary Antibacterial Defense by Cai, *et al*.**

Methods

Infection model: The *L. pneumophila* strain used in this study was Philadelphia 1 strain (ATCC 33152). The bacterium was grown on buffered charcoal-yeast extract (BCYE) agar for 2 days at 37 °C. Bacteria were harvested by centrifugation, washed twice in sterile isotonic saline, and resuspended in saline at a concentration of 20×10^7 CFU/ml. Mice were anesthetized with i.p. ketamine/xylazine and the trachea was exposed through a midventral incision followed by intratracheal inoculation of 50 µl of bacteria (10^7 CFU/50 µl/mouse). The neck incision was closed with sterile staples. Control mice were inoculated intratracheally in a similar manner with 50 µl of saline. The initial mouse inoculums were confirmed by plating serial 10-fold dilutions on BCYE agar plates.

Histopathology: Following infection, lungs were perfused from the right ventricle of heart with 10 ml of saline. Lungs were excised and fixed in 4% phosphate-buffered formalin for 18 h. Fixed lung tissues were embedded in paraffin, and 5-µm sections were cut and stained with haematoxylin and eosin. (H&E) These H&E sections were analyzed by a veterinary pathologist blinded for groups. We used the following system to score inflammation: 0, No inflammatory cells (macrophages or neutrophils) present in section; 1, <5% of section is infiltrated by inflammatory cells; 2, 5–10% of section is infiltrated by inflammatory cells; and 3, >10% of section is infiltrated by inflammatory cells. The pathological score was plotted as mean $\pm$ SE.

Figure Legends

Figure 1. IL-17A, IL-17F and IL-17A/F expression in C57BL/6 mice after *L. pneumophila* infection. **A**. C57BL/6 (WT) mice were i.t. challenged with *L. pnemophila* or saline (control) and lung homogenates were used to assess TNF-α, IL-17A, IL-17F, IL-12(p40) and IL-23(p19) mRNA levels at 6, 24, 48, and 72 h post-infection. **B**. IL-17A, IL-17F, and IL-17A/F protein levels by ELISA. Data is a representation of three individual experiments, n=4-6 mice/group; *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$ (compared with saline treated groups). Error bars represent SE.

Figure 2. Expression of IL-17A, RORγt, IL-17RA, and IL-17RC in lungs following infection. A. Infected lungs or saline challenged lungs were digested at 72 h, neutrophils were purified and stained with IL-17A Ab or control Ab. Representative dot plots show the % neutrophils expressing IL-17A and RORγt in the lungs and the bar chart shows the average + SE of 5 mice/group. **B.** BALF from infected lungs or saline-challenged lungs were collected at 72 h, neutrophils were purified and stained with IL-17A Ab or control Ab. Representative dot plots show the % neutrophils expressing IL-17A and RORγt in BALF and the bar chart shows the average + SE of 5 mice/group. **C.** BALF from infected lungs at 72 h were digested, neutrophils were purified and stained with IL-17RA Ab, IL-17RC Ab, or control Ab. Representative dot plots show the % neutrophils expressing IL-17A and RORγt in the lungs and the bar chart shows the average + SE of 5-6 mice/group (G). Error bars represent SE.

Figure 3. Expression of IFN-γ producing cells in the lungs following *L. pneumophila* infection. Flow cytometric analysis was performed on the cells obtained from whole lung digests at 24, 48, and 72 h postinfection (10^7 CFU/mouse) as described in *Materials and Methods*. Total numbers of IFN-γ+ T cells, neutrophils, and macrophages from each lung following saline challenge or infection. For experiments A-B, a total of 5-8 mice/group were used. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$ (compared with 24 h *legionella* infected mice). Error bars represent SE.

Figure 4. IL-17A is upstream of IFN-γ during *L. pneumophila* infection. Lung CFU (A) and spleen CFU (B) in *Ifng*$^{-/-}$ mice that received BSA or 1 mg recombinant murine IL-17A 1 hour prior to infection (n = 7 mice/group). *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$ (compared with 24 h *legionella* infected mice). Error bars represent SE.

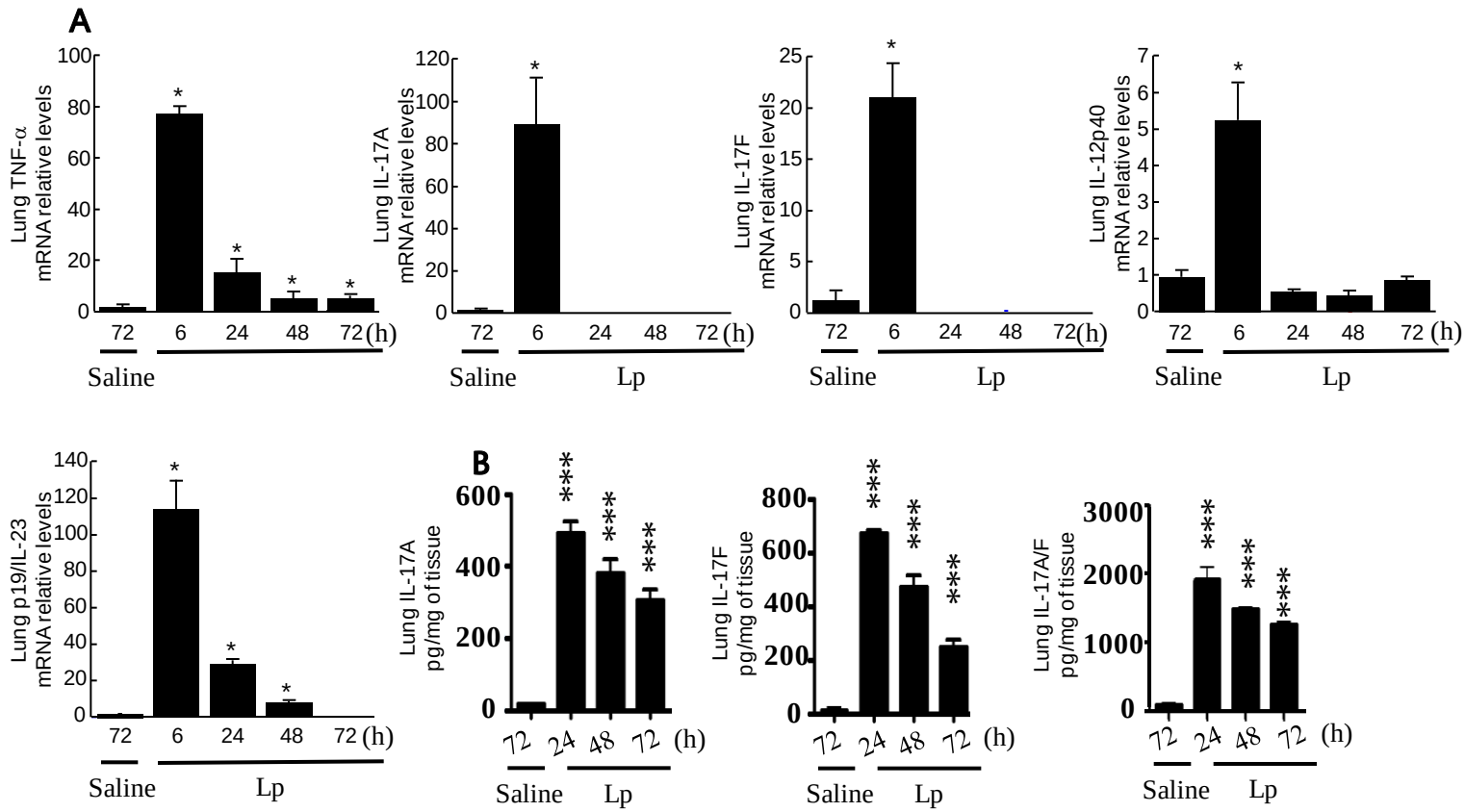
A
Lung TNF-α mRNA relative levels
Lung IL-17A mRNA relative levels
Lung IL-17F mRNA relative levels
Lung IL-12p40 mRNA relative levels
Lung p19/IL-23 mRNA relative levels
72 6 24 48 72 (h)
Saline
Lp
B
Lung IL-17A pg/mg of tissue
Lung IL-17F pg/mg of tissue
Lung IL-17A/F pg/mg of tissue
72 24 48 72 (h)
Saline
Lp

Fig. S1

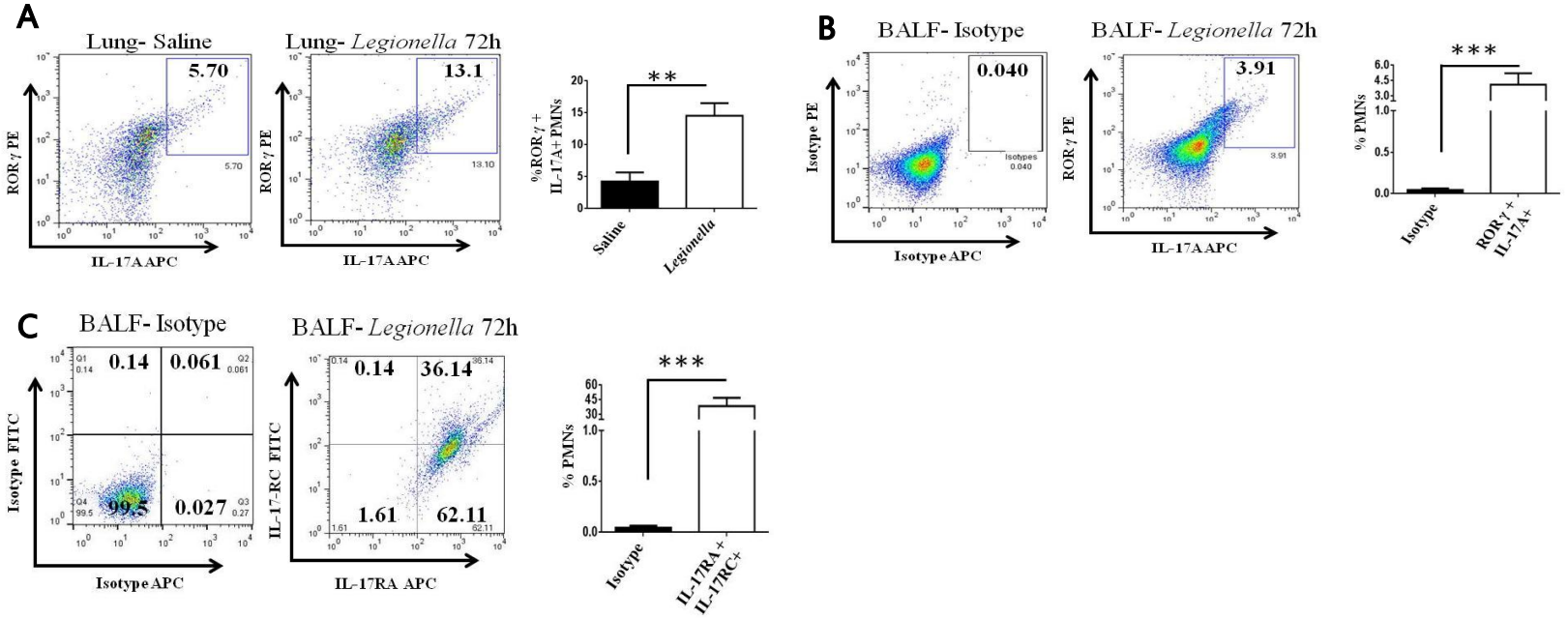
A
Lung- Saline
5.70
ROR γ PE
IL-17A APC
Lung- Legionella 72h
13.1
ROR γ PE
IL-17A APC
**
%ROR γ + IL-17A+ PMNs
Saline
Legionella
B
BALF- Isotype
0.040
Isotype PE
Isotype APC
BALF- Legionella 72h
3.91
ROR γ PE
IL-17A APC
***
% PMNs
Isotype
ROR γ + IL-17A+
C
BALF- Isotype
0.14
0.061
99.5
0.027
Isotype FITC
Isotype APC
BALF- Legionella 72h
0.14
36.14
1.61
62.11
IL-17-RC FITC
IL-17RA APC
***
% PMNs
Isotype
IL-17RA+ IL-17RC+

Fig. S2

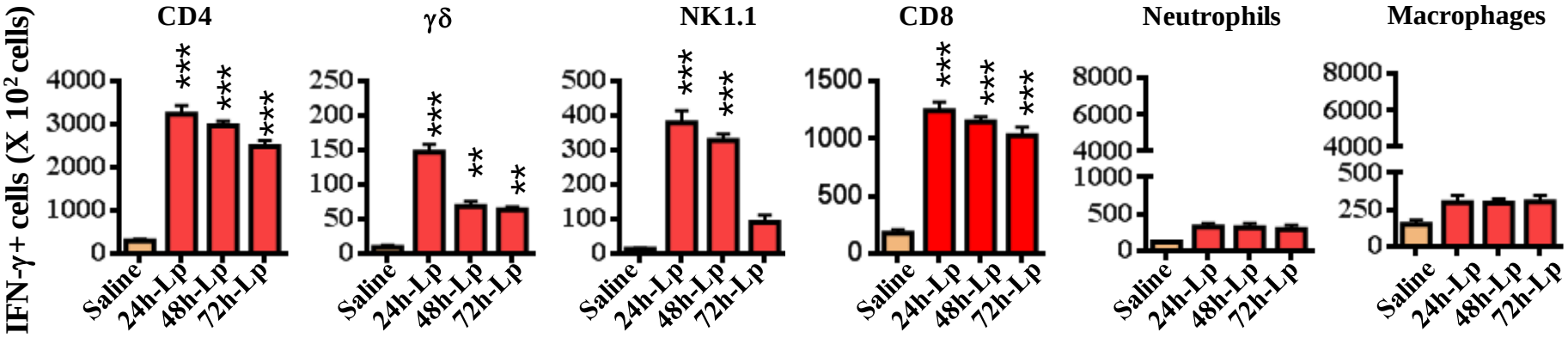
IFN-γ+ cells (X 10²cells)
CD4
γδ
NK1.1
CD8
Neutrophils
Macrophages
Saline
24h-Lp
48h-Lp
72h-Lp
***
**

Fig. S3

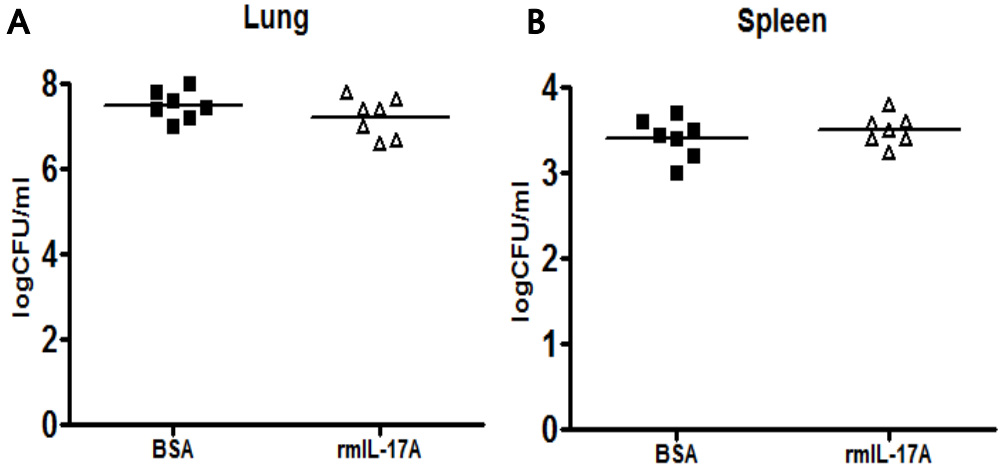
A
Lung
logCFU/ml
8
6
4
2
0
BSA
rmIL-17A
B
Spleen
logCFU/ml
4
3
2
1
0
BSA
rmIL-17A

Fig. S4