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8	Supplementary Information for
9	NLRP6 Modulates Neutrophil Homeostasis in Bacterial Pneumonia-Derived Sepsis
10	Cai et al.,
11	Corresponding author: Samithamby Jeyaseelan (jey@lsu.edu)
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Supplemental Figure 1. NLRP6 regulates the production of cytokines/chemokines in Kp-pneumonia-induced sepsis. (A-F) $Nlrp6^{-/-}$ and WT mice were infected with Kp (10^3 CFUs/mouse) and BALF and lungs were harvested at 24 and 48 h post-infection. Levels of MCP1 (A), TNF- α (B), IL-6 (C), IL-17A (D), G-CSF (E), and IL-1 β (F) in BALF or lung homogenates were measured. (n = 4-6 mice/infection group, n = 3 mice/control group) Statistical significance was determined by unpaired t-test. *, p<0.05; **, p<0.01;***, p<0.001. Error bars represent SEM.

Supplemental Fig 1

B

E 600

Kp

pg/ml

MCP-1

IL-17A

48 h

24 h

A

D

Κp

TNF-α

G-CSF

48 h

24 h

C₆₀₀.

m/bd 200

F 2500

Kp

IL-6 ***

IL-1β <u>**</u>

48 h

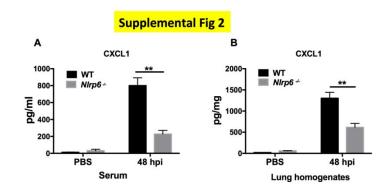
24 h

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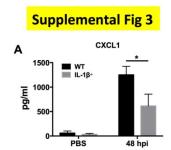
43 44

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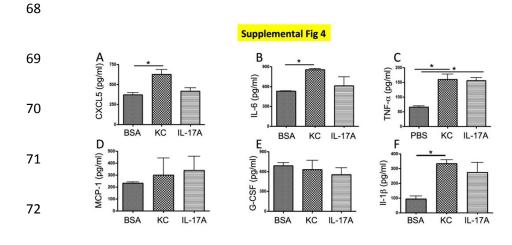


Supplemental Figure 2. Level of CXCL1 in Kp-infected *Nlrp6*- $^{-}$ and WT mice. (A-B) *Nlrp6*- $^{-}$ and WT mice were infected with Kp (10³ CFUs/mouse). CXCL1 were measured in serum and lung homogenates. (n = 4-6 mice/infection group, n = 3 mice/control group) Statistical

significance was determined by unpaired t-test. **, p<0.01. Error bars represent SEM.



Supplemental Figure 3. Abundance of CXCL1 in Kp-infected IL-1 $\beta^{-/-}$ and WT mice. (A) IL- $I\beta^{-/-}$ and WT mice were infected with Kp (10³ CFUs/mouse) and CXCL1 were measured in the BALF. (n = 4–6 mice/infection group, n = 3 mice/control group) Statistical significance obtained by unpaired t-test. *, p<0.05. Error bars represent SEM.



Supplemental Figure 4. Production of cytokines/chemokines in Kp-pneumonia-induced sepsis in mice receiving rII-17A or rCXCL1. (A-F) $Nlrp6^{-/-}$ mice were infected with Kp (10³ CFU/mouse) with or without administration of rCXCL1 or rIL-17A or BSA at the time of infection. Levels of CXCL5 (A), IL-6 (B), TNF- α (C), MCP-1 (D), G-CSF (E), and IL-1 β (F) in BALF were measured. (n = 4–6 mice/group) Statistical significance was determined by ANOVA (followed by Tukey's multiple comparision). *, p<0.05. Error bars represent SEM.