Supplemental Methods:

BMDM culture and experiments. Tibias and femurs were flushed with PBS to collect bone marrow cells. Cells were RBC-lysed using ACK lysis buffer, washed, and suspended in the DMEM containing 10% FBS and Penicillin/Streptomycin (100U/ml). Undifferentiated bone marrow cells were set up in culture dishes by supplementing MCSF (50 ng/ml) on days 0, 2, 4, 6 for a week until they become macrophages. TLR agonists (Pam2CSK4, Pam3CSK4, HKSA, HKSP, LTA, and LPS) used in BMDM assays were purchased from InvivoGen. Inhibitors of p38 (SB203580), JNK (SP600125), ERK (PD098059), and NF-κB were purchased from Calbiochem.

Cytokine and chemokine production. Mouse CXCL1, CXCL2, CXCL5 (R&D Systems) and CXCL12 (eBioscience) were quantified according to manufacturers' protocols.

Bone marrow chimeras. In brief, recipient mice were lethally irradiated with a single dose of 1000 rad. RBC-lysed BM cells (4 x10^6/mouse) were injected into each irradiated mouse through tail vein injection. BM-transfused mice were kept on 0.2% neomycin sulfate drinking water for 2 weeks and rested for 2 months before inducing pneumonia. Our routine procedures resulted in least 75 - 85% of the reconstitution of blood leukocytes as observed through GFP-expressing donor cells.

Flow cytometry for granulopoiesis. In brief, bone marrow cells that had lost the potential to give rise to granulocytes were removed from the target population (Figure 3A). The remaining cells were then analyzed for expression of c-Kit and Ly-6G (Figure 3A). First, cells expressing lineage marker for T cells (CD3, CD4, CD8), B cells (B220), and erythroid cells (Ter119) are gated out along with dead cells (R1 gate). Second, the

population of $SSC^{high}FSC^{int}$ cells in R2 gate representing eosinophils is removed too from further analysis. Next, within R3 gate, when analyzed for the expression of CD34 and c-Kit, population of cells in R4 gate representing megakaryocyte–erythroid progenitors (MEP) ($c\text{-}Kit^{high}CD34^{low}$ cells) are also removed from further analysis. Finally, the remaining cells (R5) were then analyzed for the expression of c-Kit and Ly6G and divided into five cell subpopulations (#1–#5). The subpopulations #1 ($c\text{-}Kit^{high}Ly6G^{neg}$), #2 ($c\text{-}Kit^{int}Ly6G^{neg}$), #3 ($c\text{-}Kit^{int\text{-}neg}Ly6G^{neg\text{-}int}$) represent early granulocytic compartments, whereas subpopulation #4 ($c\text{-}Kit^{int\text{-}neg}Ly6G^{int}$) and #5 ($c\text{-}Kit^{neg}Ly6G^{high}$) are immature to mature neutrophils ready to be released.

Supplemental Figure Legends:

Supplemental Figure 1. Dose- and time-dependent CXCL1 production by pneumococci. (A) WT BMDMs were infected with *S. pneumoniae* 6303 (MOI 1-50) or PBS for 2 to 18 hours. Supernatants were collected at indicated time points and CXCL1 was measured.

Supplemental Figure 2. CXCL1 differentially regulates recruitment and homeostasis of immune cells. (A) WT and *Cxcl1*$^{-/-}$ mice were infected with *S. pneumoniae* 6303 (5 x 10^4 CFU) or PBS. Mice were sacrificed at 48 hours post-infection and lungs were collected. Representative FACs plot and histograms of immune cells. (n= 5-6 mice/infection group, n=3 mice/control group). All experiments were performed three times. Statistical significance was determined by unpaired t-test. *p<0.05; **p<0.01.

Supplemental Figure 3. Neutrophil recruitment and bacterial clearance in *Cxcl1*$^{-/-}$ mice with A66.1, WU2, and D39 pneumonia-induced sepsis. (A-C) WT and *Cxcl1*$^{-/-}$ mice were infected with *S. pneumoniae* 2 x 10^5 CFUs for A66.1, 5 x10^7 CFU for WU2,

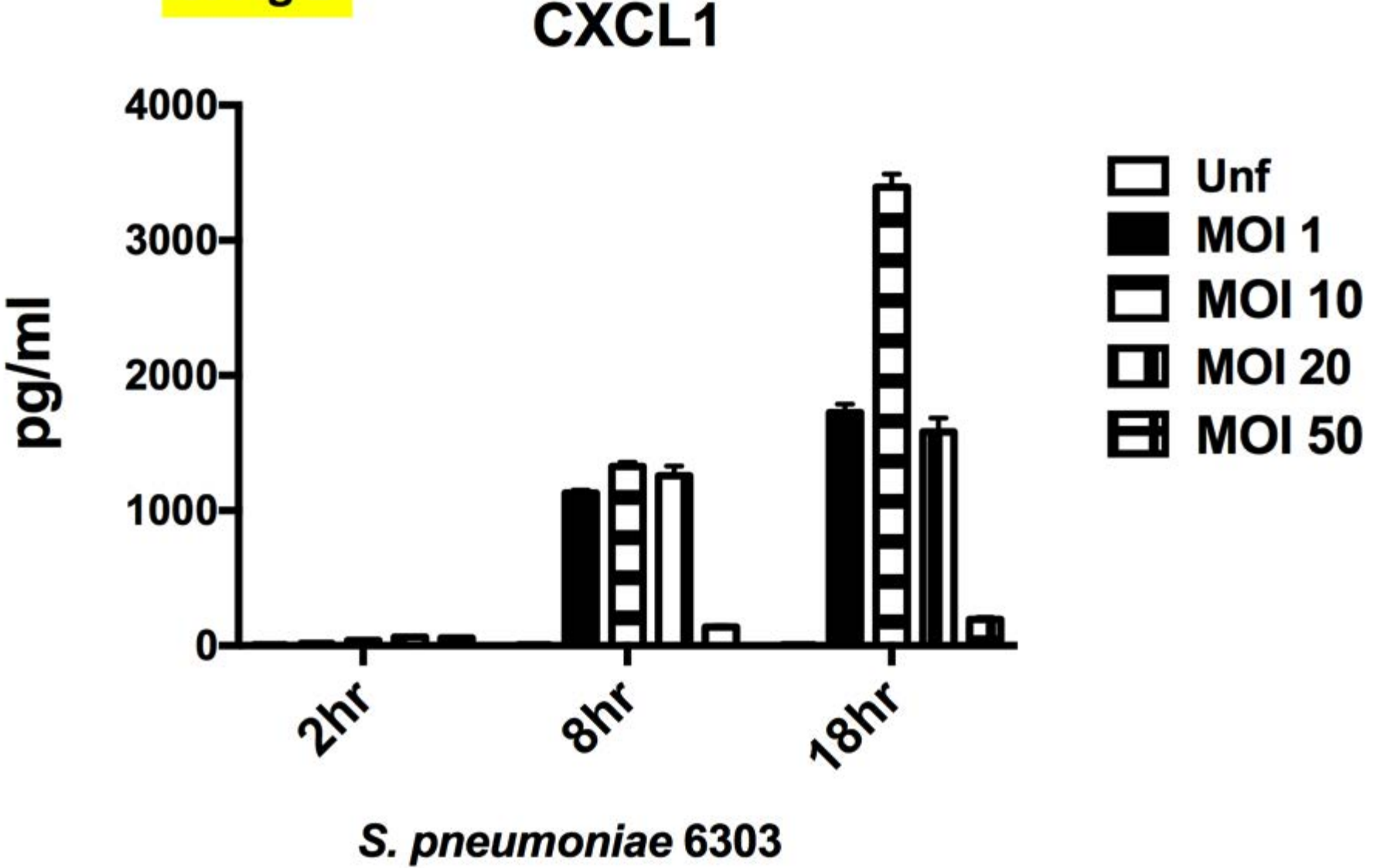
and 5 x10^4 CFU for D39 or PBS respectively. Mice were sacrificed at 48-hours post infection, and BALF and lungs were collected. Neutrophils counts in BALF and bacterial burden in BALF and lungs were enumerated. (n= 5-6 mice/infection group, n=3 mice/control group). All experiments were performed three times. Statistical significance was determined by unpaired t-test. *p<0.05; **p<0.01.

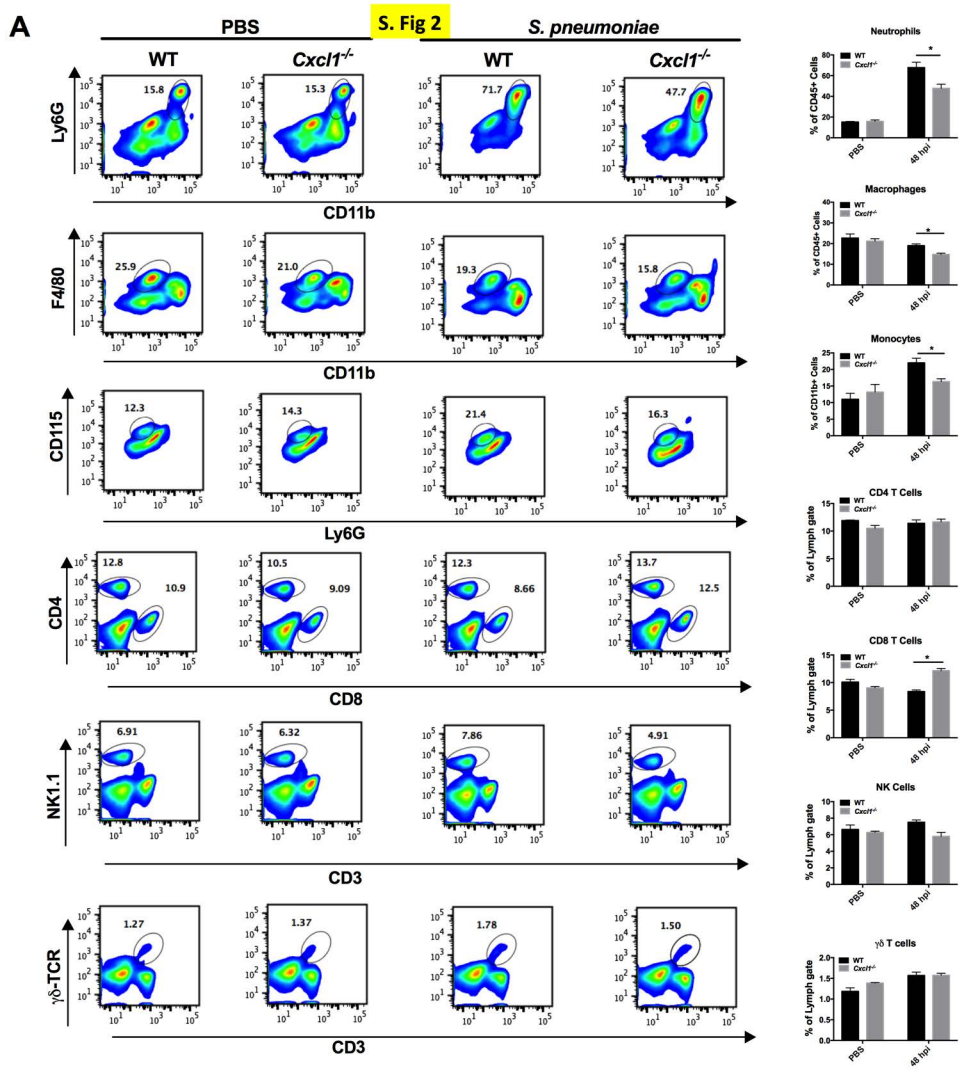
Supplemental Figure 4. Expressions of integrins and L-selectins in bone marrow in pneumonia-induced sepsis. (A) WT and *Cxcl1*$^{-/-}$ mice were infected with *S. pneumoniae* 6303 5 x10^4 CFU or PBS. Mice were sacrificed at 48 hours post-infection, and BM cells were collected. Representative histograms and MFIs of the CD11a, CD11b, CD18, and CD29 were enumerated in BM neutrophils. **(B)** WT mice were infected with *S. pneumoniae* 6303 5 x10^4 CFU or PBS. Mice were treated with L-selectin sheddase inhibitor (TAPI-O or KD-IX-73-4) or vehicle control (DMSO) i.p. at 0 and 24 post infection and then sacrificed at 48 hours post-infection and BM cells were collected. Representative histograms and MFIs of the CD62L were enumerated in BM neutrophils. (n= 5-6 mice/infection group, n=3 mice/control group). All experiments were performed three times. Statistical significance was determined by unpaired t-test. *p<0.05.

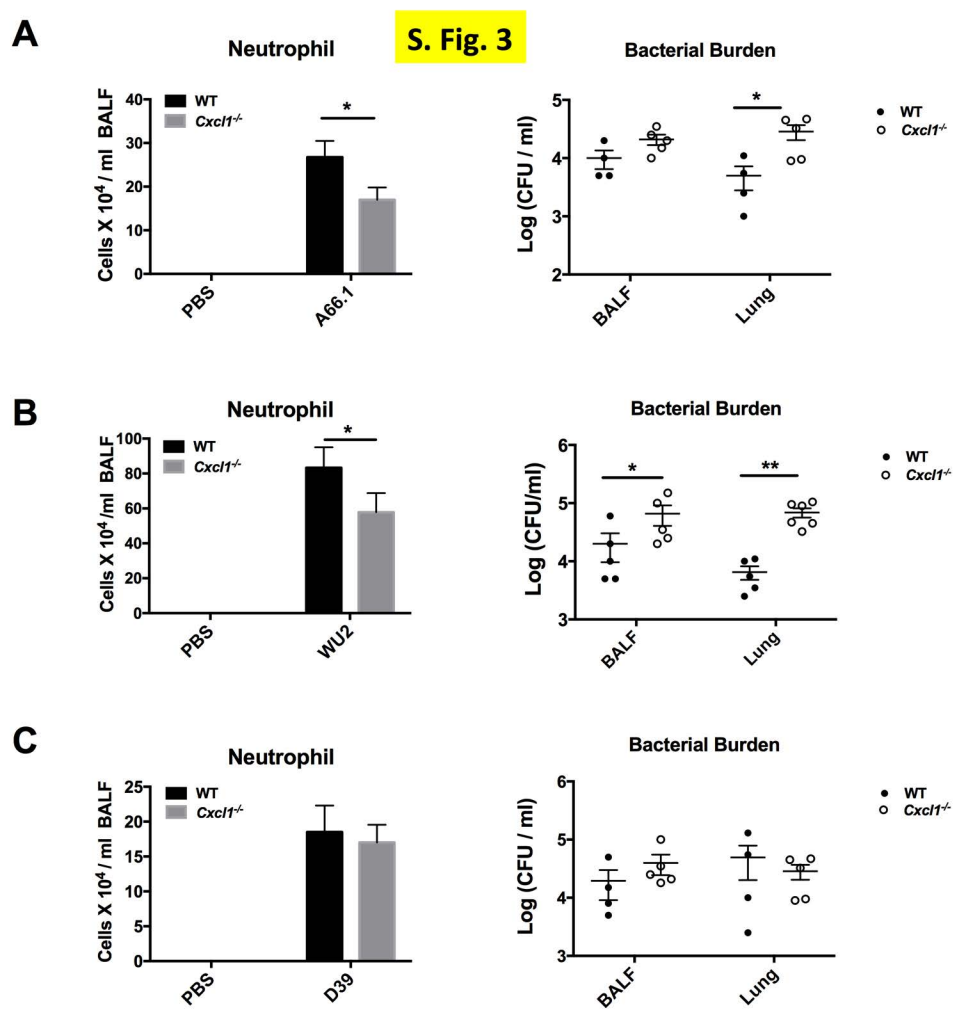
Supplemental Figure 5. CXCL1 regulates CXCL2 and CXCL5 production in pneumococcal pneumonia-induced sepsis (A) WT and *Cxcl1*$^{-/-}$ mice were infected with *S. pneumoniae* 6303 (5 x 10^^{4} CFU) or PBS. Mice were sacrificed at 48 hours post-infection, BALF was collected, and CXCL2 and CXCL5 were measured. (n= 5-6 mice/infection group, n=3 mice/control group). All experiments were performed three times. Statistical significance was determined by unpaired-test. **p<0.01.

Supplemental Figure 6. Neutralization of CXCL2 and CXCL5 in pneumococcal pneumonia-induced sepsis (A) WT mice were treated with either anti-CXCL2 or anti-CXCL5 or both i.p. at 24 and 2 hours prior to infection and then infected intratracheally with *S. pneumoniae* 6303 (5 X 10^4 CFU). Control groups received IgG. FACS dot plot **(A)**, and number per femur/tibia **(B)** of subpopulations #5 within the granulopoietic compartment, FACS dot plot **(C)**, and number per femur/tibia **(D)** of myeloid progenitor cells (c-Kit$^+$Sca-1$^-$Lin$^-$), and FACS dot plot **(E)**, and percentage **(F)** of blood neutrophils at 48-hours post infection are presented. (n= 5-6 mice/infection group, n=3 mice/control group). Statistical significance was determined by one-way ANOVA (followed by Bonferroni's *post hoc* comparisons) **p*<0.05; ***p*<0.01.

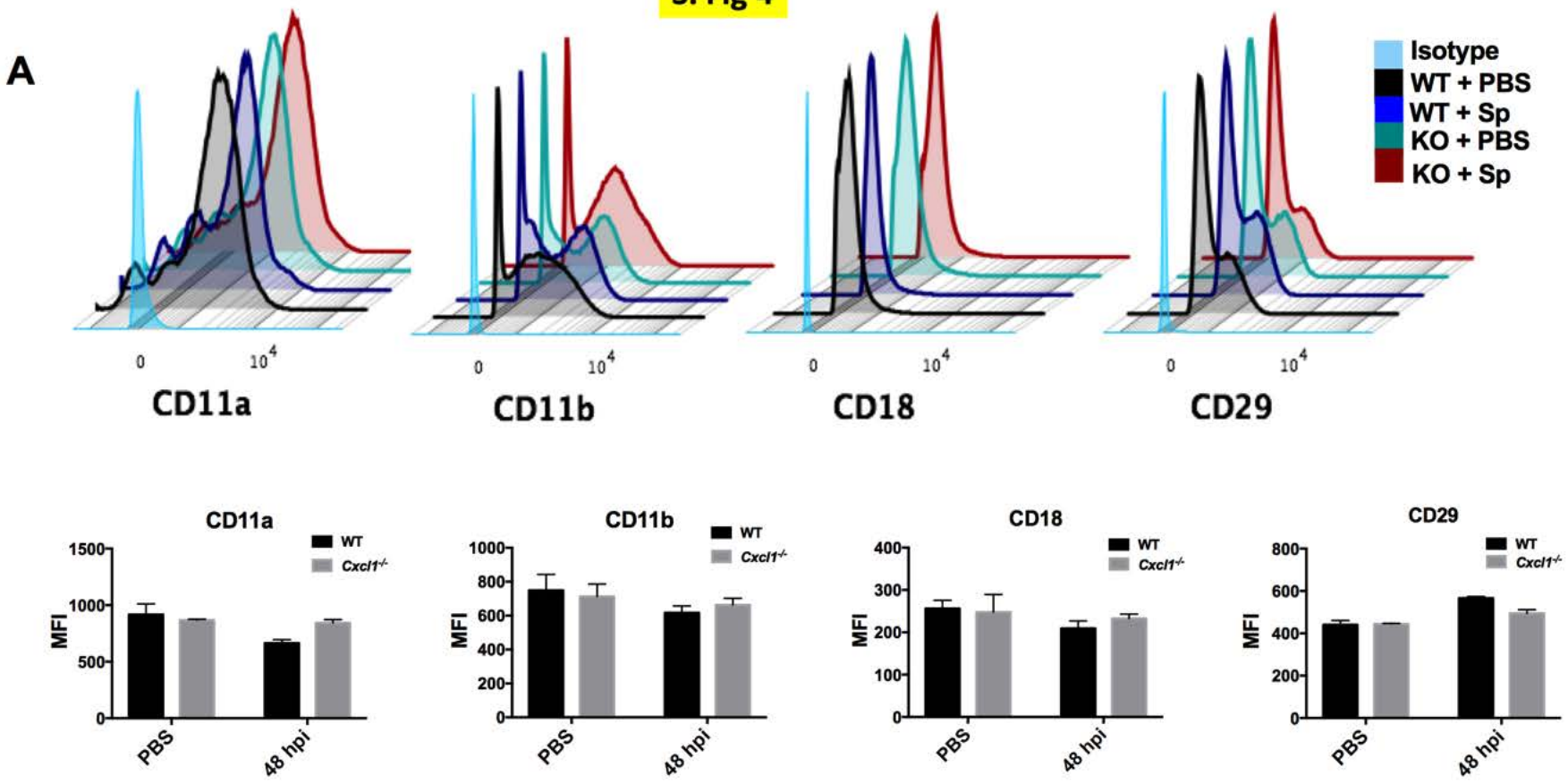
S. Fig 1


CXCL1
4000
3000
2000
1000
0
pg/ml
2hr
8hr
18hr
Unf
MOI 1
MOI 10
MOI 20
MOI 50
S. pneumoniae 6303


A
S. Fig 2
PBS
S. pneumoniae
WT
Cxcl1-/-
WT
Cxcl1-/-
Ly6G
CD11b
15.8
15.3
71.7
47.7
F4/80
CD11b
25.9
21.0
19.3
15.8
CD115
Ly6G
12.3
14.3
21.4
16.3
CD4
CD8
12.8
10.9
10.5
9.09
12.3
8.66
13.7
12.5
NK1.1
CD3
6.91
6.32
7.86
4.91
γδ-TCR
CD3
1.27
1.37
1.78
1.50
Neutrophils
% of CD45+ Cells
Macrophages
% of CD45+ Cells
Monocytes
% of CD11b+ Cells
CD4 T Cells
% of Lymph gate
CD8 T Cells
% of Lymph gate
NK Cells
% of Lymph gate
γδ T cells
% of Lymph gate
PBS
48 hpi
*

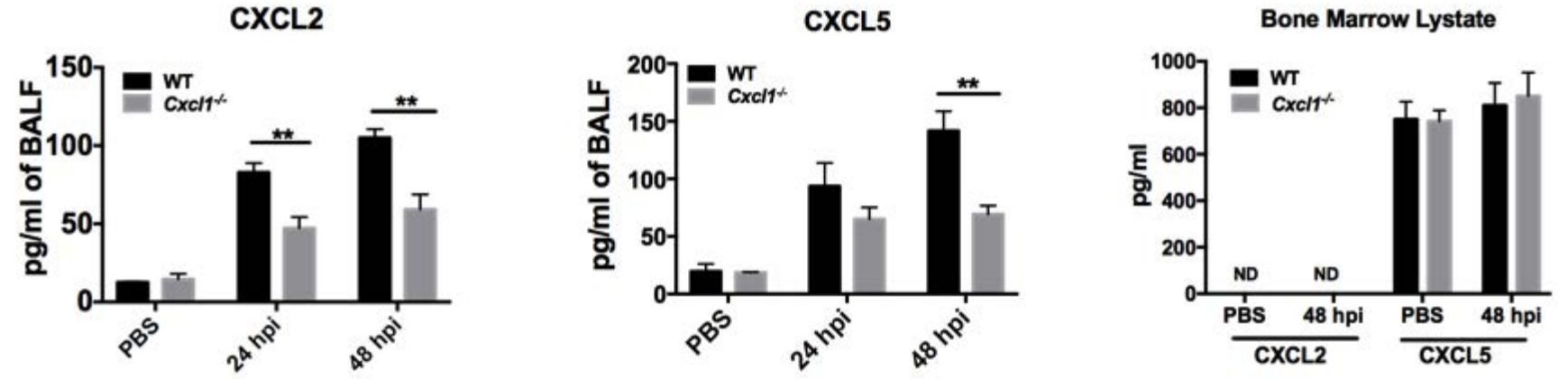

A
Neutrophil
S. Fig. 3
Bacterial Burden
Cells X 10^4 / ml BALF
WT
Cxcl1-/-
PBS
A66.1
Log (CFU / ml)
BALF
Lung
B
Neutrophil
Bacterial Burden
Cells X 10^4 /ml BALF
PBS
WU2
Log (CFU/ml)
BALF
Lung
C
Neutrophil
Bacterial Burden
Cells X 10^4 / ml BALF
PBS
D39
Log (CFU / ml)
BALF
Lung

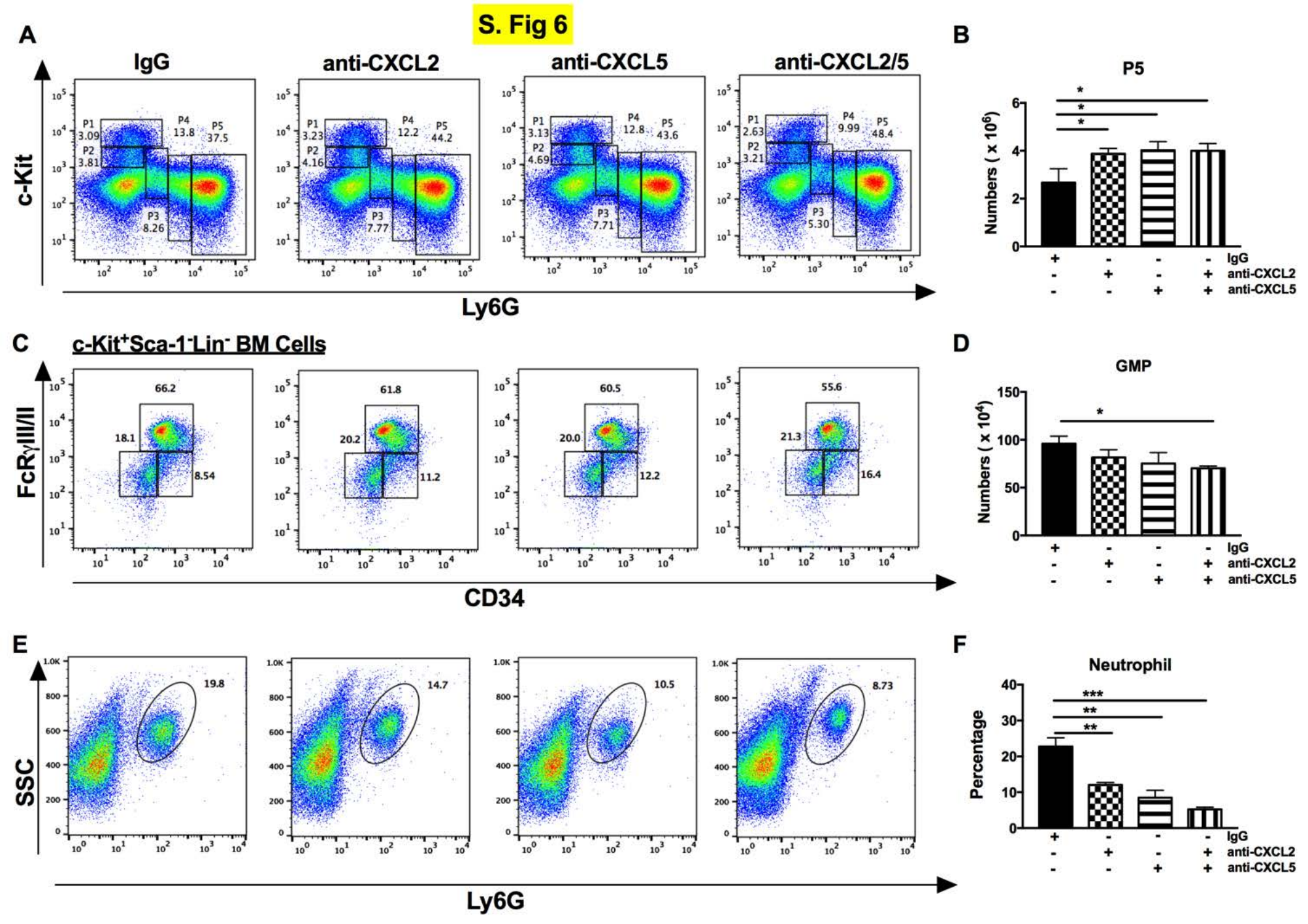
S. Fig 4


A
Isotype
WT + PBS
WT + Sp
KO + PBS
KO + Sp
0
10^4
CD11a
CD11b
CD18
CD29
CD11a
CD11b
CD18
CD29
WT
Cxcl1-/-
MFI
PBS
48 hpi
B
Isotype
WT + DMSO
WT + TAPI-O
-10^3
0
10^3
10^4
10^5
CD62L
CD62L
DMSO
TAPI-O
*
MFI

S. Fig 5

A


CXCL2
pg/ml of BALF
150
100
50
0
WT
Cxcl1-/-
**
**
PBS
24 hpi
48 hpi
CXCL5
pg/ml of BALF
200
150
100
50
0
WT
Cxcl1-/-
**
PBS
24 hpi
48 hpi
Bone Marrow Lystate
pg/ml
1000
800
600
400
200
0
WT
Cxcl1-/-
ND
ND
PBS
48 hpi
PBS
48 hpi
CXCL2
CXCL5


S. Fig 6
A
IgG
anti-CXCL2
anti-CXCL5
anti-CXCL2/5
c-Kit
Ly6G
P1 3.09
P2 3.81
P3 8.26
P4 13.8
P5 37.5
P1 3.23
P2 4.16
P3 7.77
P4 12.2
P5 44.2
P1 3.13
P2 4.69
P3 7.71
P4 12.8
P5 43.6
P1 2.63
P2 3.21
P3 5.30
P4 9.99
P5 48.4
B
P5
Numbers (x 10^6)
IgG
anti-CXCL2
anti-CXCL5
C
c-Kit+Sca-1-Lin- BM Cells
FcRγIII/II
CD34
66.2
18.1
8.54
61.8
20.2
11.2
60.5
20.0
12.2
55.6
21.3
16.4
D
GMP
Numbers (x 10^4)
E
SSC
Ly6G
19.8
14.7
10.5
8.73
F
Neutrophil
Percentage