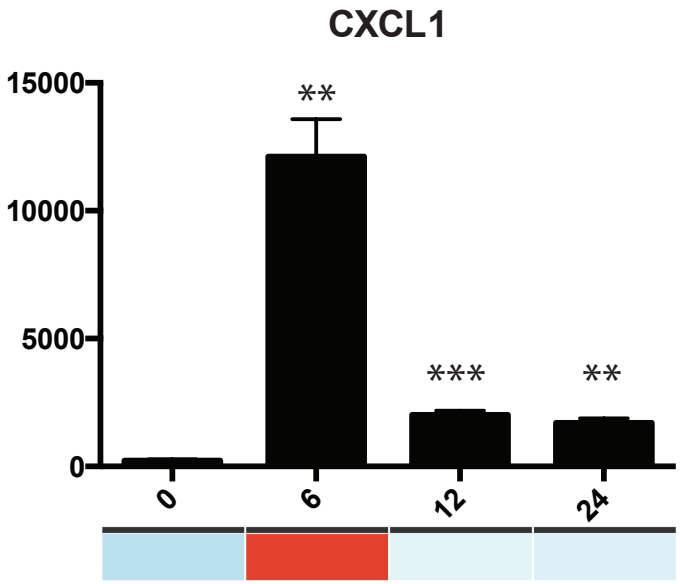
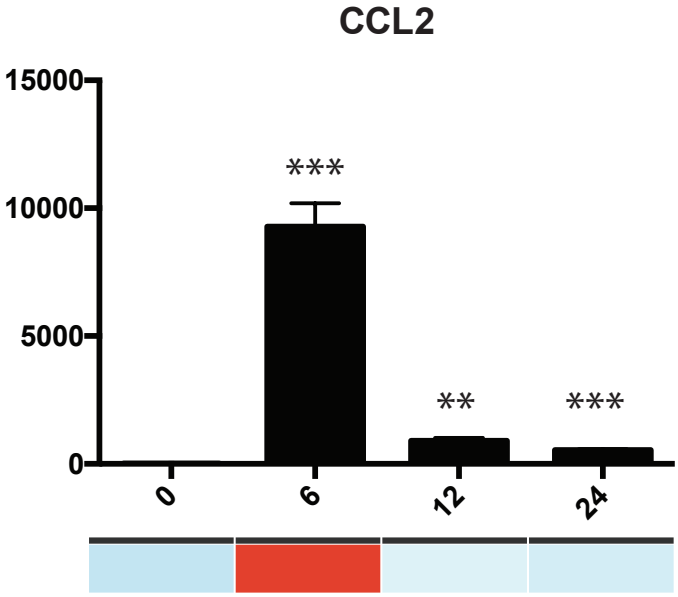
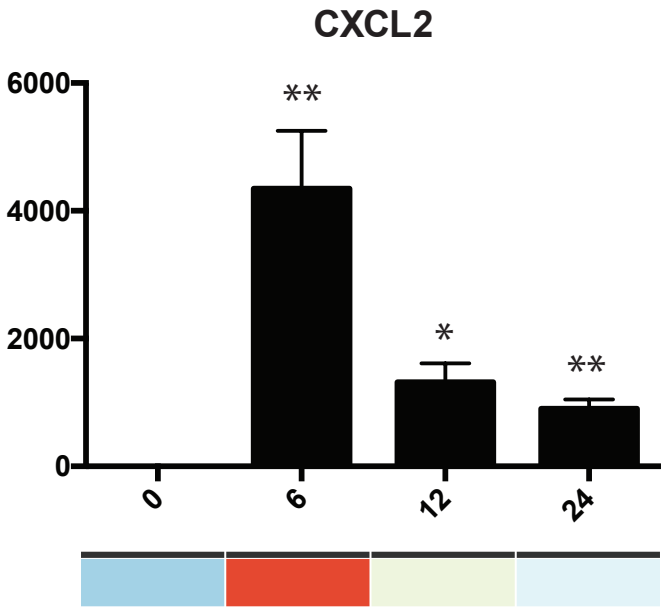
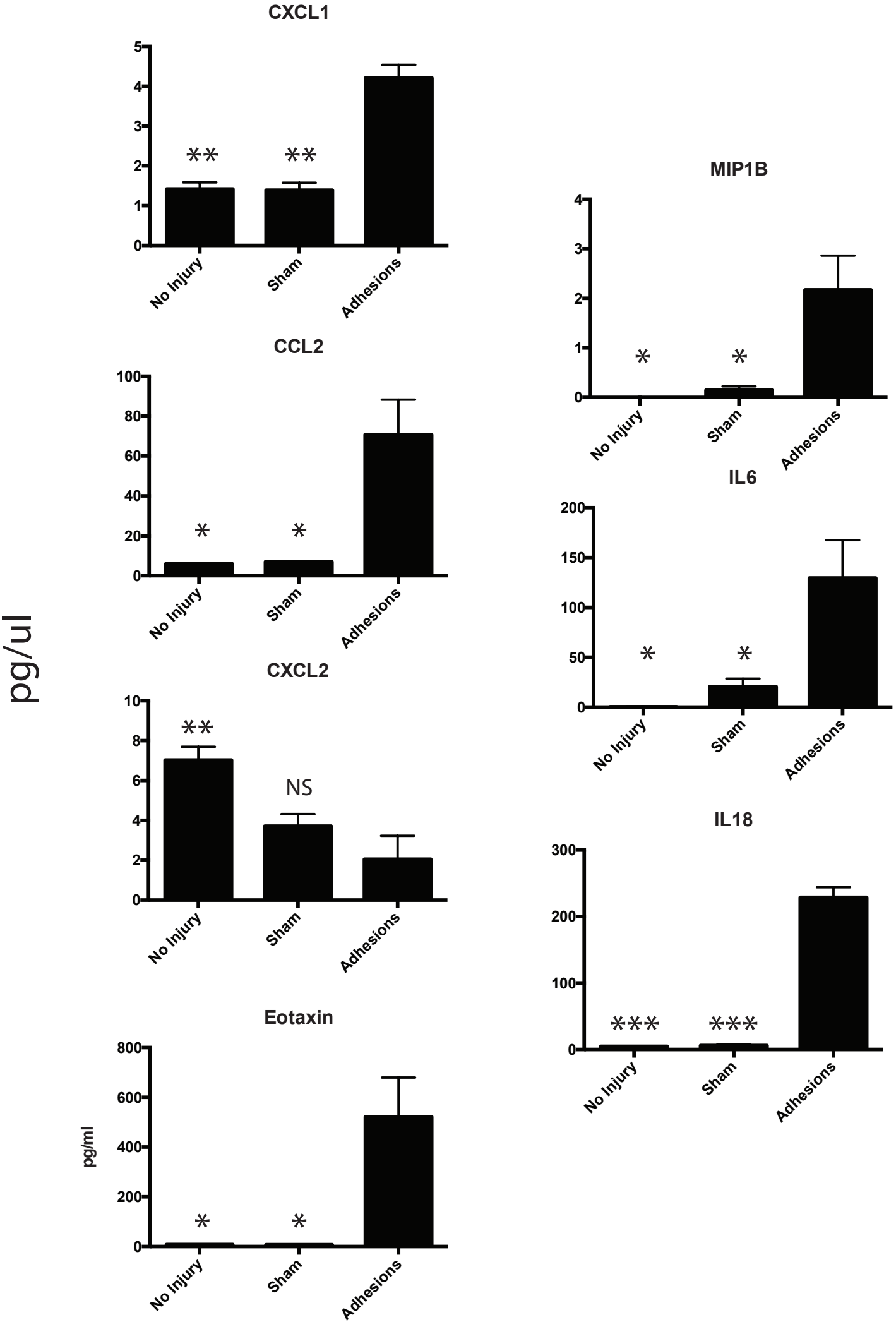

CXCL1
15000
10000
5000
0
**
***
**
0
6
12
24


CCL2
15000
10000
5000
0
***
**
***
0
6
12
24


CXCL2
6000
4000
2000
0
**
*
**
0
6
12
24

Supplementary Figure 1: Mesothelial cytokine transcript levels during adhesion formation

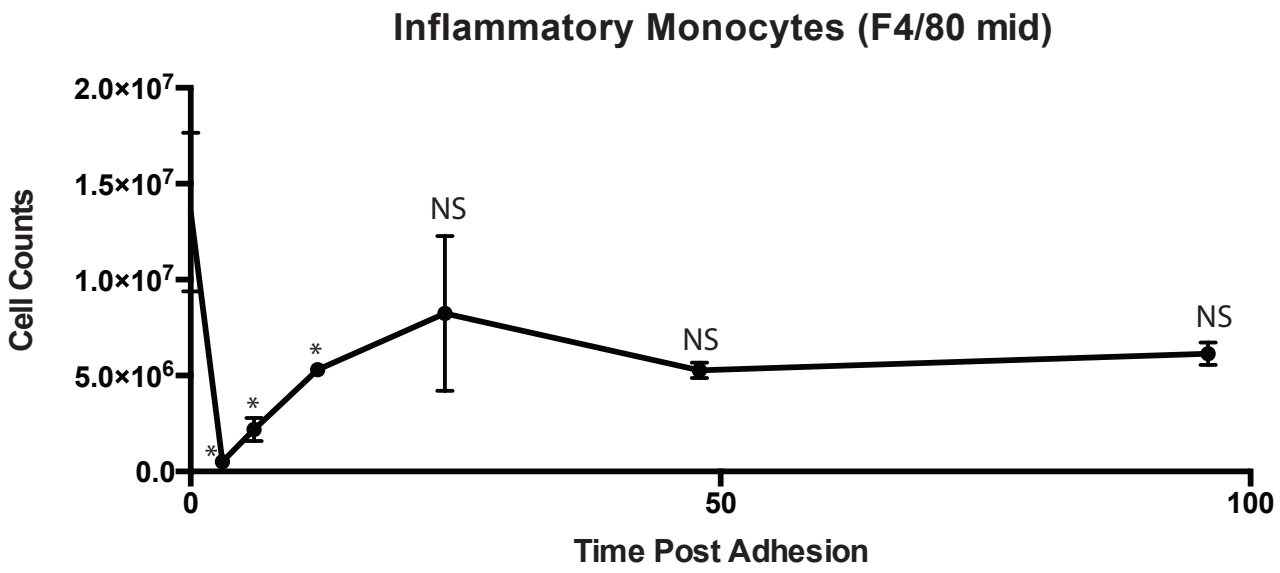
Transcript expression levels (FPKM) of CXCL1, MCP-1, and CXCL2 from injured mesothelium 0, 6, 12, and 24 hours post button placement. Error bars are SEM; *$p<0.05$, **$p<0.005$, ***$p<0.0005$.


CXCL1
5
4
3
2
1
0
**
**
No Injury
Sham
Adhesions
MIP1B
4
3
2
1
0
*
*
No Injury
Sham
Adhesions
CCL2
100
80
60
40
20
0
*
*
No Injury
Sham
Adhesions
IL6
200
150
100
50
0
*
*
No Injury
Sham
Adhesions
CXCL2
10
8
6
4
2
0
**
NS
No Injury
Sham
Adhesions
IL18
300
200
100
0
***
***
No Injury
Sham
Adhesions
Eotaxin
pg/ml
800
600
400
200
0
*
*
No Injury
Sham
Adhesions
pg/ul

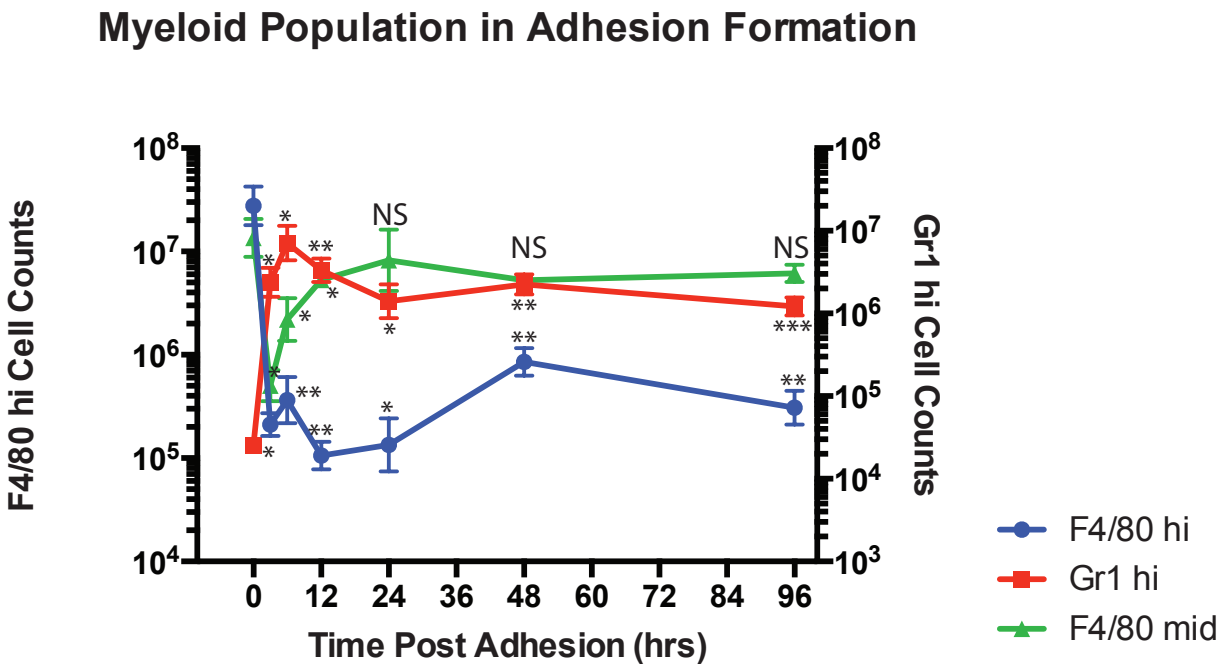
Supplementary Figure 2: Mesothelial cytokine secretion during adhesion formation

Protein levels (pg/ul) of cytokines in uninjured mice and mice receiving either sham (peritoneal lapratomy and closure) or adhesion surgeries. Error bars are SEM; *$p<0.05$, **$p<0.005$, ***$p<0.0005$; NS = not significant.

A

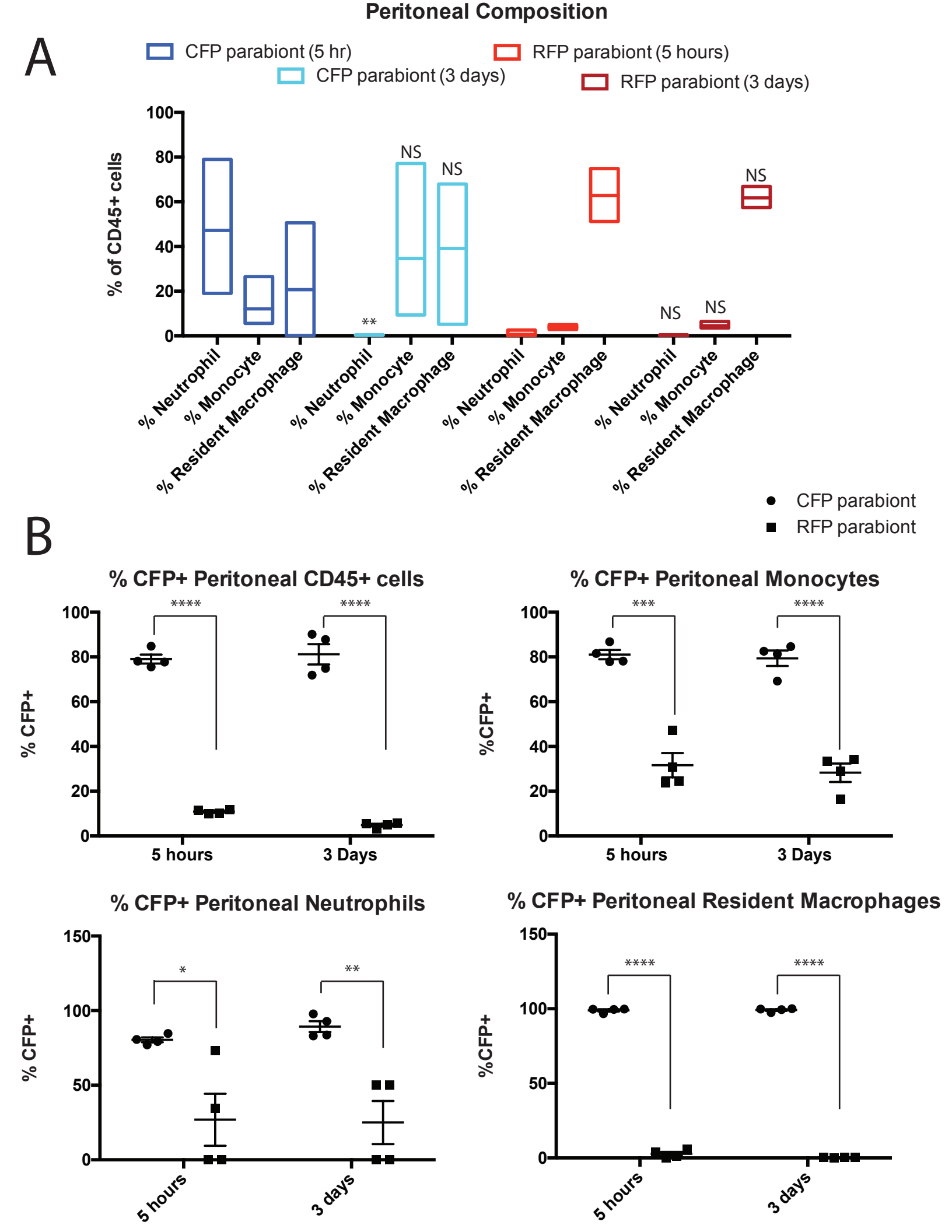

Inflammatory Monocytes (F4/80 mid)
Cell Counts
2.0×10^7
1.5×10^7
1.0×10^7
5.0×10^6
0.0
0
50
100
Time Post Adhesion
NS
NS
NS
*
*
*
*

B


Myeloid Population in Adhesion Formation
F4/80 hi Cell Counts
Gr1 hi Cell Counts
10^8
10^7
10^6
10^5
10^4
10^3
0
12
24
36
48
60
72
84
96
Time Post Adhesion (hrs)
NS
NS
NS
F4/80 hi
Gr1 hi
F4/80 mid

Supplementary Figure 3: Inflammatory Monocytes Infiltrate into the Peritoneum Following Injury

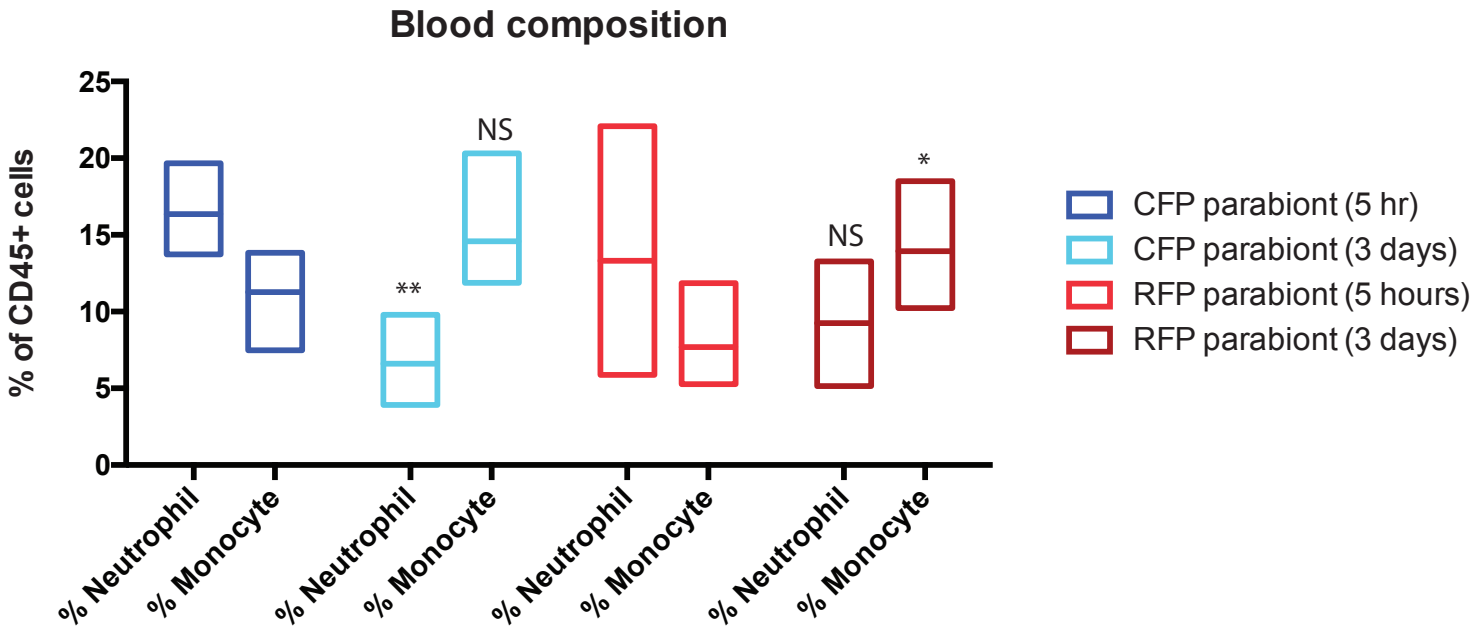
A Characterization of myeloid population from 0 to 96 hours following adhesion formation: Absolute numbers of inflammatory monocytes (CD11b+F4/80mid) were analyzed at each time point. **B** Absolute numbers of inflammatory monocytes (CD11b+F4/80mid), tissue resident macrophages (CD11b+F4/80hi), and neutrophils (Gr1+) at each time point. *$p<0.05$, **$p<0.005$, ***$p<0.0005$; NS = not significant.


Peritoneal Composition
A
CFP parabiont (5 hr)
CFP parabiont (3 days)
RFP parabiont (5 hours)
RFP parabiont (3 days)
% of CD45+ cells
100
80
60
40
20
0
NS
NS
**
NS
NS
NS
% Neutrophil
% Monocyte
% Resident Macrophage
B
CFP parabiont
RFP parabiont
% CFP+ Peritoneal CD45+ cells
% CFP+
****
****
5 hours
3 Days
% CFP+ Peritoneal Monocytes
%CFP+
***
****
5 hours
3 Days
% CFP+ Peritoneal Neutrophils
% CFP+
150
100
50
0
*
**
5 hours
3 days
% CFP+ Peritoneal Resident Macrophages
%CFP+
****
****
5 hours
3 days

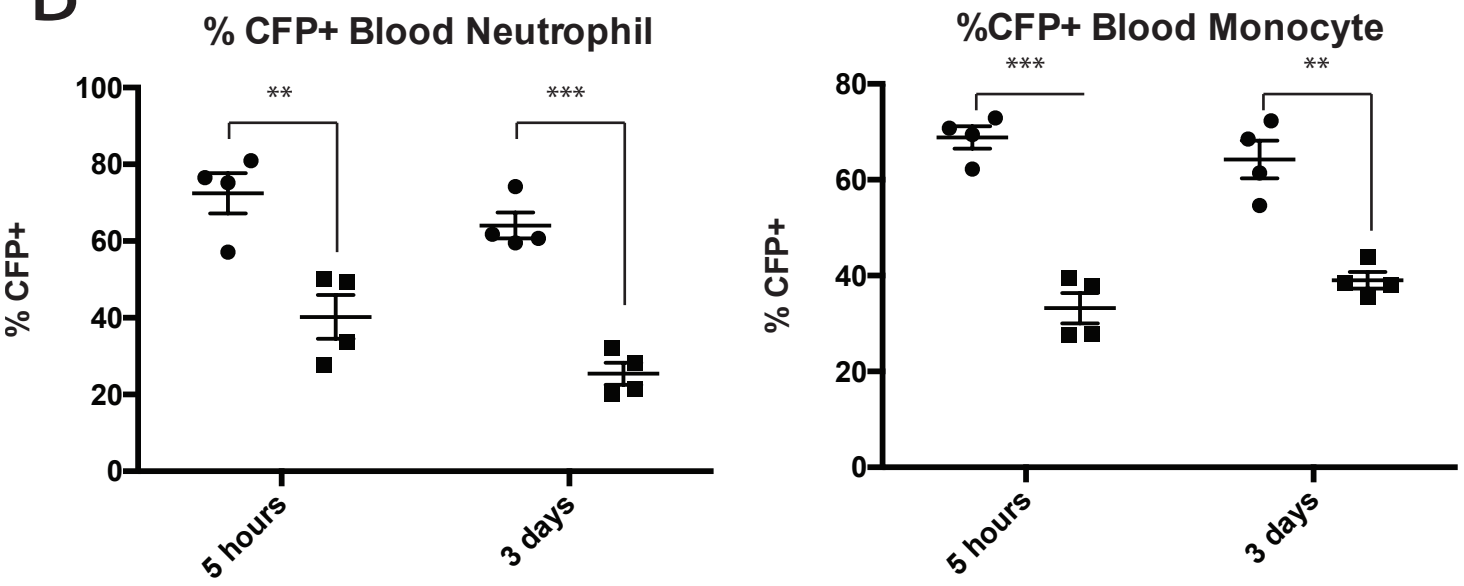
Supplementary Figure 4: Myeloid composition of peritoneum following thioglycollate injection in parabiotic mice

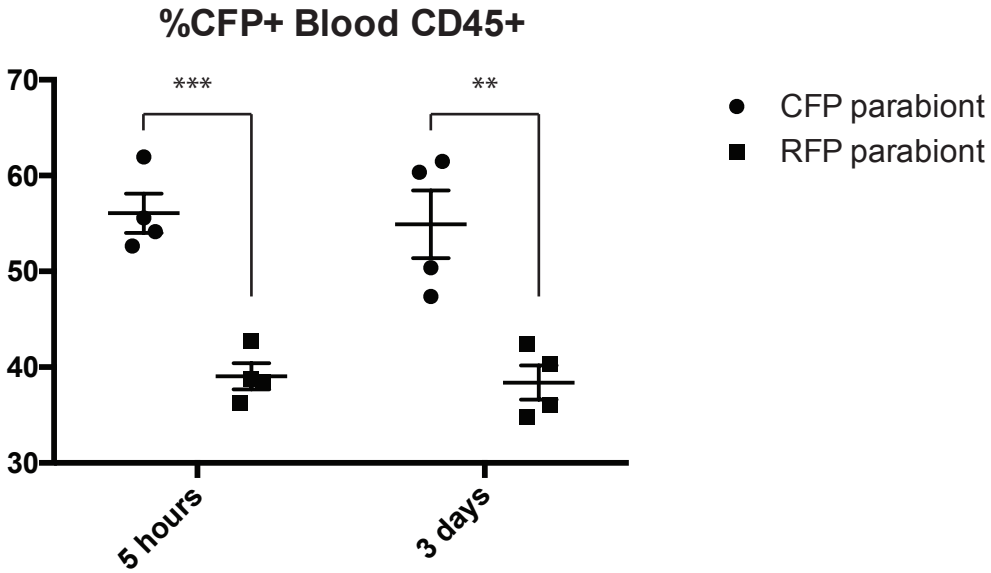
A Percent neutrophils, monocyte, or macrophage of total CD45+ cells in the peritoneum 5 hours or 3 days following thioglycollate administration in parabiotic mice. **B** Percent CFP+ neutrophils, macrophages, or monocytes in CFP mouse from the parabiotic pair 5 hours or 3 days following thioglycollate administration. *$p<0.05$, **$p<0.005$, ***$p<0.0005$; NS = not significant.

A


Blood composition
% of CD45+ cells
25
20
15
10
5
0
**
NS
NS
*
% Neutrophil
% Monocyte
% Neutrophil
% Monocyte
% Neutrophil
% Monocyte
% Neutrophil
% Monocyte
CFP parabiont (5 hr)
CFP parabiont (3 days)
RFP parabiont (5 hours)
RFP parabiont (3 days)

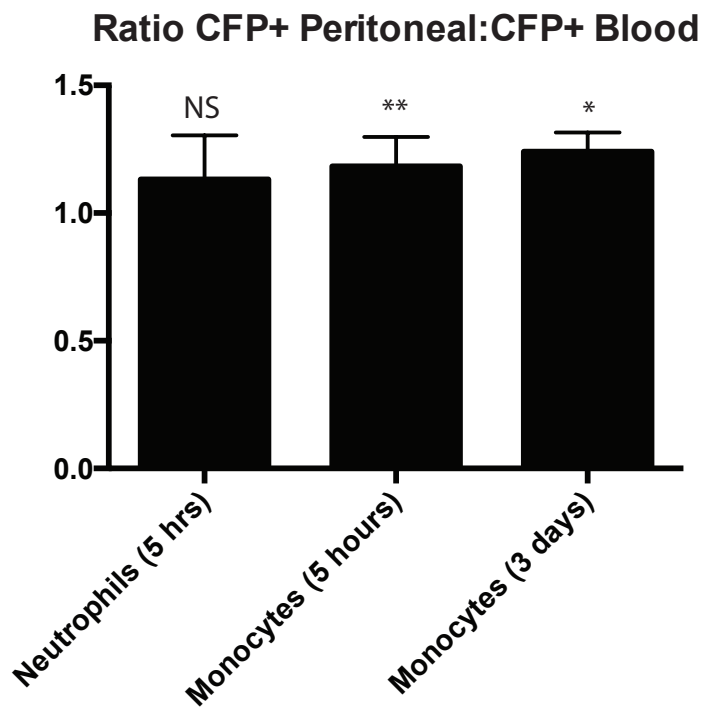
B


% CFP+ Blood Neutrophil
% CFP+
100
80
60
40
20
0
**
***
5 hours
3 days
%CFP+ Blood Monocyte
% CFP+
80
60
40
20
0
***
**
5 hours
3 days


%CFP+ Blood CD45+
70
60
50
40
30
***
**
5 hours
3 days
CFP parabiont
RFP parabiont

Supplementary Figure 5: Myeloid composition of blood following thioglycollate injection in parabiotic mice

A Percent neutrophils or monocyte of total CD45+ cells 5 hours or 3 days in circulation following thioglycollate administration in parabiotic mice. **B** Percent CFP+ neutrophils, macrophages, or monocytes in CFP mouse from the parabiotic pair 5 hours or 3 days following thioglycollate administration. *$p<0.05$, **$p<0.005$, ***$p<0.0005$; NS = not significant.

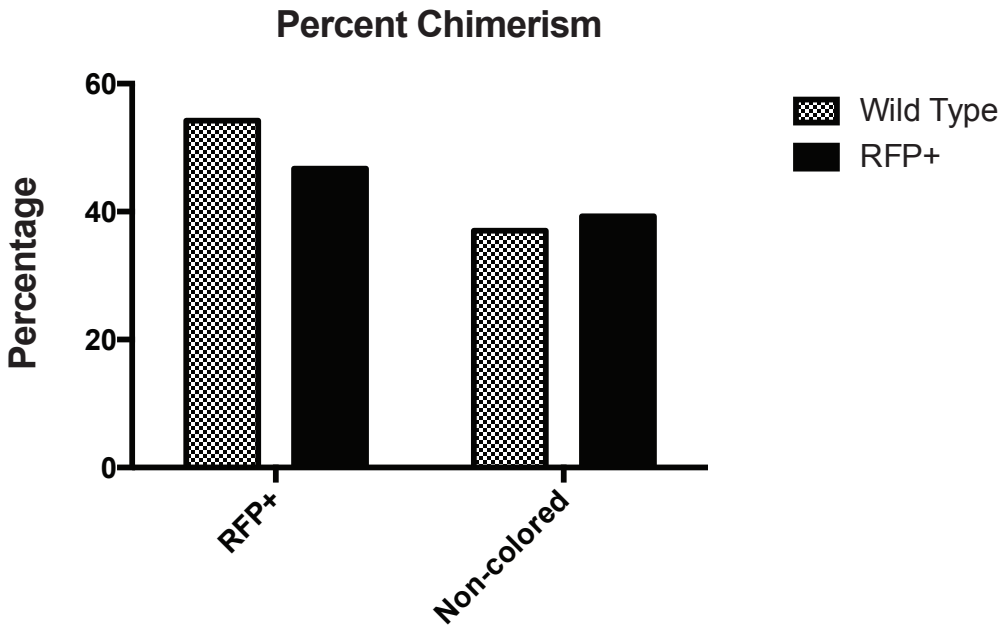


Cell type CFP chimerism enrichment in peritoneum over blood

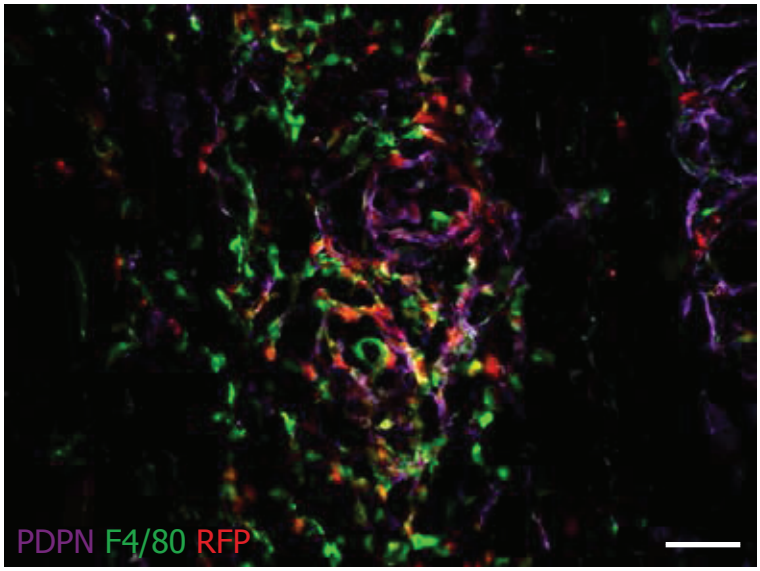
Supplementary Figure 6: Relative distribution of myeloid compartment in blood and peritoneum

Ratio of CFP+ neutrophils or monocytes in peritoneum versus blood. *p<0.05, **p<0.005, ***p<0.0005; NS = not significant.

A

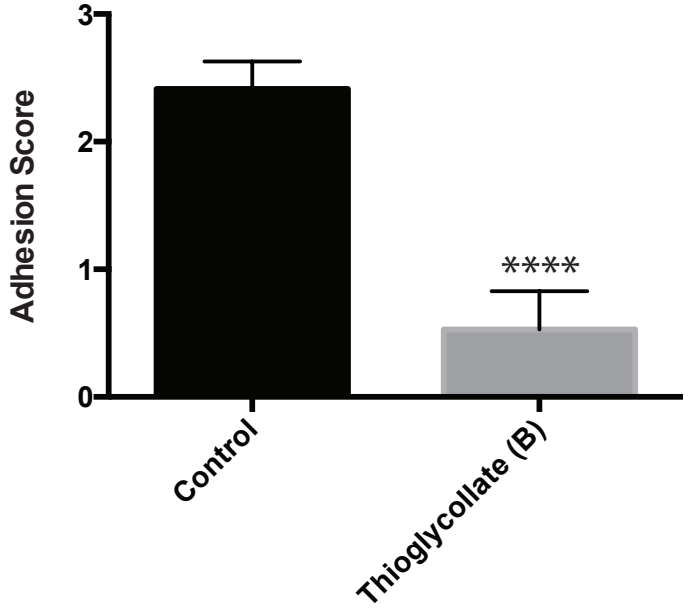

Percent Chimerism
Percentage
60
40
20
0
RFP+
Non-colored
Wild Type
RFP+

B


PDPN F4/80 RFP

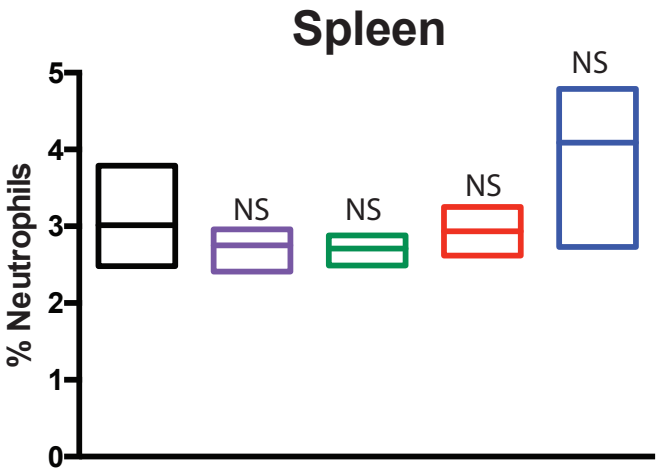
Supplementary Figure 7: Parabiotic mice suggest circulating inflammatory cells contribute to adhesion formation

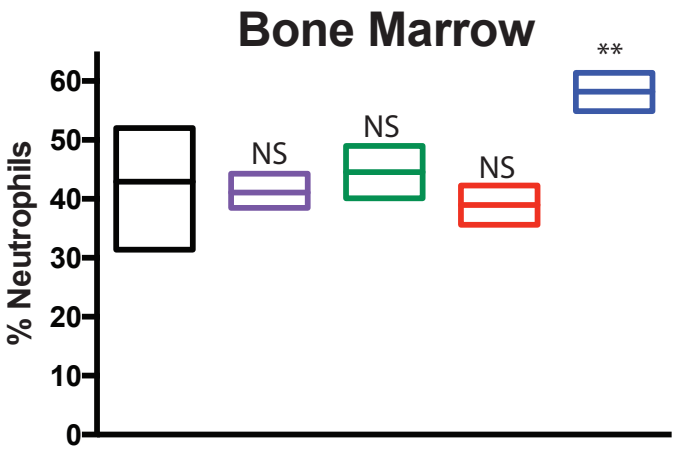
A Blood from wild type, red parabionts was analyzed via flow cytometry for chimerism (percent uncolored and red cells in each parabiont). **B** Composite immunofluorescence staining for PDPN and F4/80 of adhesions from wild type mice of a wild type / RFP^+ parabionts pair. All scale bars are 100 μm.

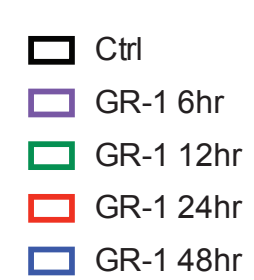

Adhesion Score
3
2
1
0
****
Control
Thioglycollate (B)

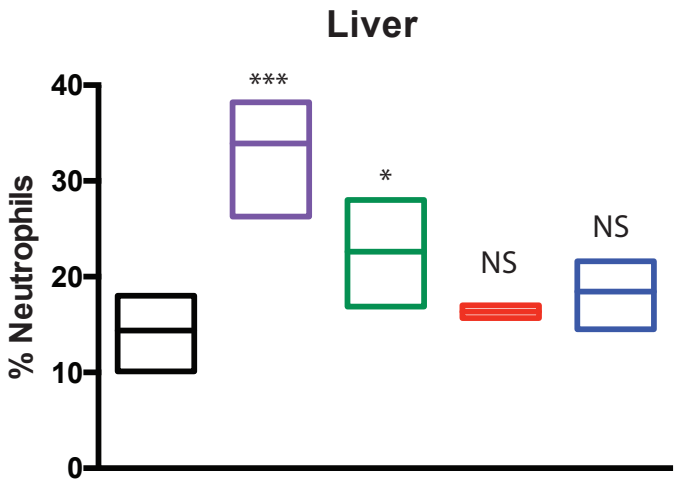
Supplementary Figure 8: Thioglycollate administration significantly reduces adhesion formation

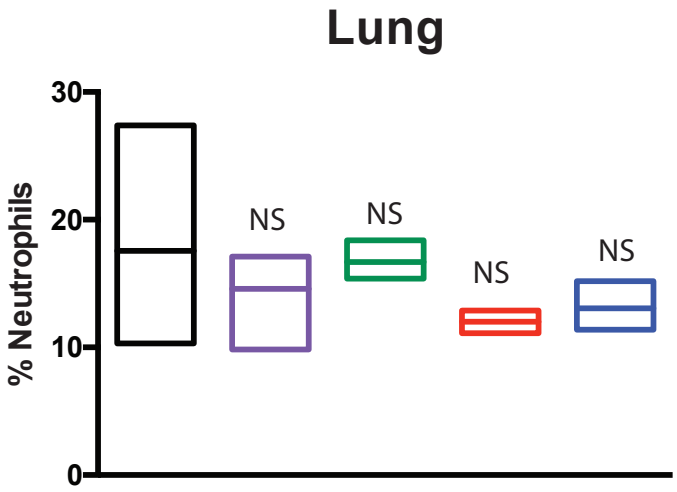
Adhesion scoring of adhesion induced mice treated with thioglycollate (n = 17) versus vehicle controls (n = 63). ****p<0.0001; NS = not significant.

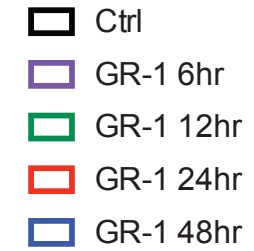

Spleen
% Neutrophils
0
1
2
3
4
5
NS
NS
NS
NS


Bone Marrow
% Neutrophils
0
10
20
30
40
50
60
NS
NS
NS
**


Ctrl
GR-1 6hr
GR-1 12hr
GR-1 24hr
GR-1 48hr

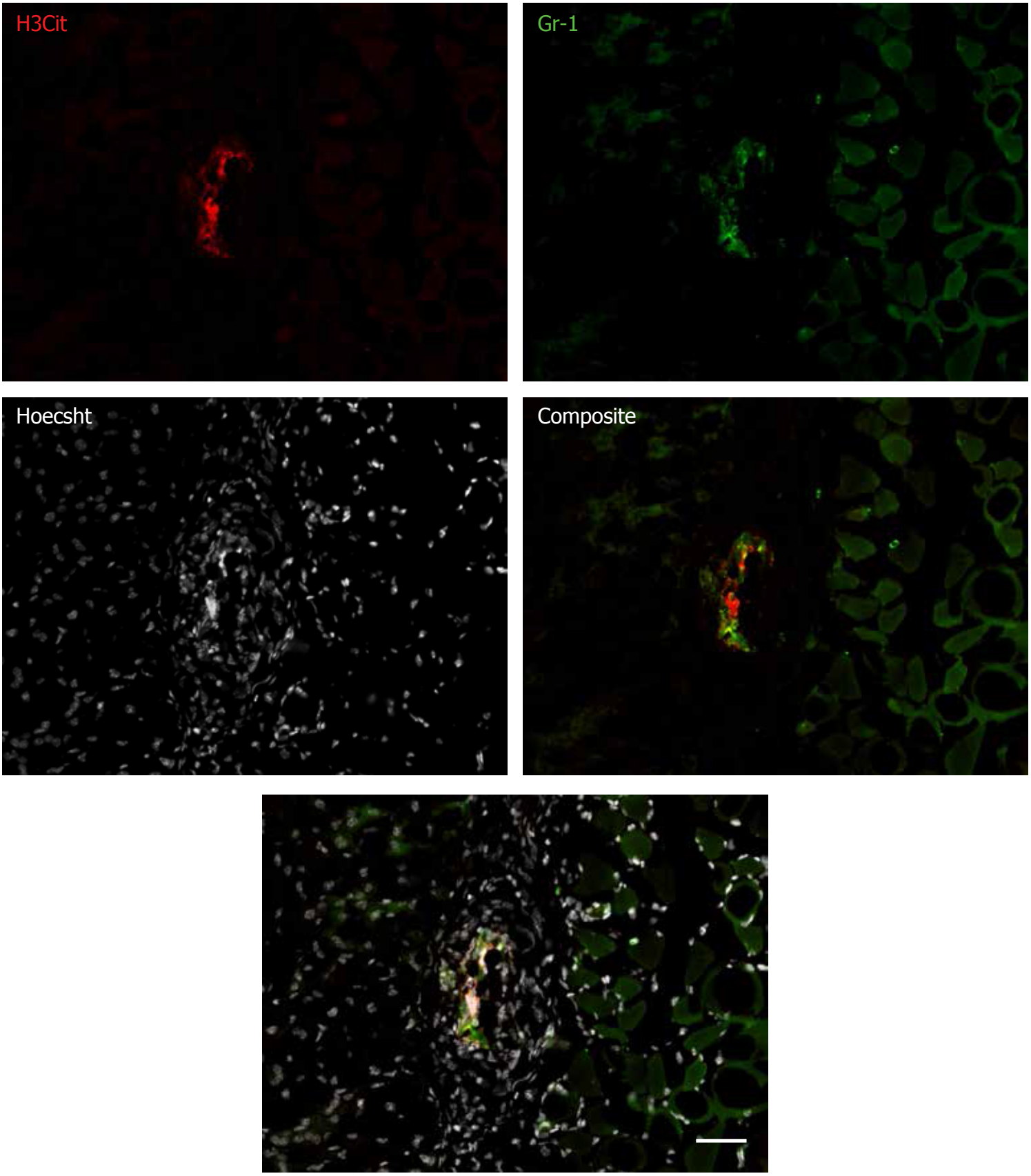

Liver
% Neutrophils
0
10
20
30
40
***
*
NS
NS


Lung
% Neutrophils
0
10
20
30
NS
NS
NS
NS


Ctrl
GR-1 6hr
GR-1 12hr
GR-1 24hr
GR-1 48hr

Supplementary Figure 9: Effect of anti-GR1 on neutrophil compartments

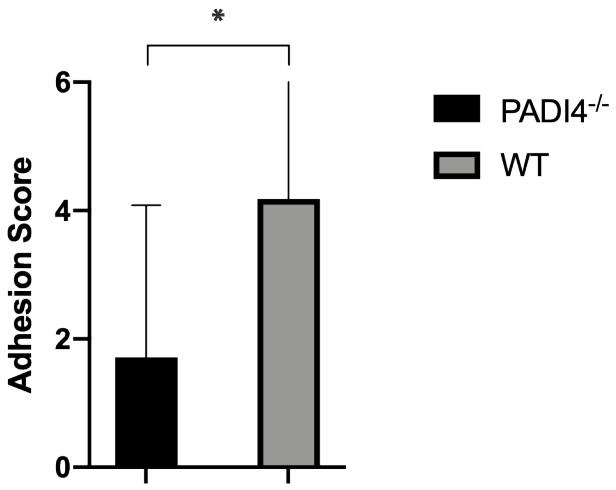
Percentage of neutrophils in spleen, bone marrow, lung, and liver 6, 12, 24, and 48 hours following anti-Gr-1 administration. *$p<0.05$, **$p<0.005$, ***$p<0.0005$; NS = not significant.


H3Cit
Gr-1
Hoecsht
Composite

Supplementary Figure 10: NETs play a role in adhesion induction and are associated with GR1+ cells

Single and composite immunofluorescence staining for H3Cit, Gr-1, and Hoescht in adhesion tissue 7 days following adhesion induction. All scale bars are 100 μm.

Adhesion Severity in PADI4-Deficient Mice



Supplementary Figure 11: Adhesions in PADI4$^{-/-}$ Mice are less severe

Adhesion scoring of adhesion induced in PADI4 $^{-/-}$ mice (n = 14) versus controls (n = 11). *$p<0.05$.