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.
Supplementary Figure 2: Mesothelial cytokine secretion during adhesion formation

Protein levels (pg/ul) of cytokines in uninjured mice and mice receiving either sham (peritoneal laparotomy 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

0.0
5.0×10^6
1.0×10^7
1.5×10^7
2.0×10^7

Time Post Adhesion

B

Myeloid Population in Adhesion Formation

F4/80 hi Cell Counts

Gr1 hi Cell Counts

Time Post Adhesion (hrs)

0 12 24 36 48 60 72 84 96

* 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

% of CD45+ cells

% Neutrophil | % Monocyte | % Resident Macrophage | % Neutrophil | % Monocyte | % Resident Macrophage | % Neutrophil | % Monocyte | % Resident Macrophage | % Neutrophil | % Monocyte | % Resident Macrophage

0 | 20 | 40 | 60 | 80 | 100

CFP parabiont (5 hr) | RFP parabiont (5 hours) | CFP parabiont (3 days) | RFP parabiont (3 days)

B

% CFP+ Peritoneal CD45+ cells

% CFP+ Peritoneal Monocytes

% CFP+ Peritoneal Neutrophils

% CFP+ Peritoneal Resident Macrophages

<table>
<thead>
<tr>
<th>5 hours</th>
<th>3 Days</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>80</td>
<td>100</td>
</tr>
</tbody>
</table>

CFP parabiont | RFP parabiont

** NS NS NS NS NS **** **** *******

* ** **** ****
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.
**Blood composition**

- **A)**
  - Graph showing the percentage of CD45+ cells over time for different conditions.
  - Conditions: CFP parabiont (5 hr), CFP parabiont (3 days), RFP parabiont (5 hours), RFP parabiont (3 days).
  - Neutrophil and Monocyte percentages over time are compared.

- **B)**
  - Graphs showing the percentage of CFP+ Blood Neutrophil and Monocyte over time for different conditions.
  - Conditions: 5 hours, 3 days.
  - Graphs for CFP+ Blood CD45+ showing different conditions and time points.
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

- Wild Type
- RFP+

Percentage

RFP+
Non-colored

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.
Thioglycollate (B)

Adhesion Score

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: NS
- % Neutrophils: NS
- % Neutrophils: NS
- % Neutrophils: NS

**Bone Marrow**

- % Neutrophils: NS
- % Neutrophils: NS
- % Neutrophils: NS
- % Neutrophils: **

**Liver**

- % Neutrophils: ***
- % Neutrophils: *
- % Neutrophils: NS

**Lung**

- % Neutrophils: NS
- % Neutrophils: NS
- % Neutrophils: NS
- % Neutrophils: NS

Legend:
- 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.
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

![Bar graph showing adhesion score comparison between PADI4-/- and WT mice. The graph indicates a statistically significant difference (*) between the two groups.](image-url)
Supplementary Figure 11: Adhesions in PADI4<sup>−/−</sup> Mice are less severe

Adhesion scoring of adhesion induced in PADI4<sup>−/−</sup> mice (n = 14) versus controls (n = 11). *p<0.05.