Online Supplement

Supplemental Methods

Reagents and cells

Purified batroxobin, plasminogen, and factor XIIIa (fXIIIa) were obtained from Enzyme Research Laboratories (ERL). The chemotactic agent C5a was obtained from R&D systems. The RAW264.7 cell line was obtained from the ATCC and was used in low passage numbers (i.e., less than 10 passages). Transwell migration inserts with 5 µM pores were from Corning. Murine fibrinogen was purified from citrate-plasma by ammonium sulfate precipitation as described.[1] Aliquots were confirmed to be free of endotoxin contamination prior to use via ToxinSensor Chromogenic LAL assay (GenScript; minimum detection limit, 0.005 EU/mL). Primary bone marrow-derived macrophages (BMDMs) were generated from wildtype mice as described.[2]

Immunohistochemistry

Immunohistochemistry was performed as in fibrin immunofluorescence with slight modifications. Sections were incubated with rat anti-F4/80 antibody (BM8, ThermoFisher Scientific MF48000, 1:500) O/N at 4°C and washed in PBS (3 times). Secondary antibody (Biotinylated goat anti-rat IgG, Vector Laboratories Inc. BA-9400, 1:500) was added for 1 hour at room temperature. Sections were then washed, developed, dehydrated and #1.5 cover slips mounted with ProLong™ Gold Antifade Reagent (Thermo Fisher Scientific). Images were captured on an Olympus BX43 Clinical upright microscope (40x) using Olympus DP74 color camera. A fixed square area was selected around the tissue and the color deconvolution plugin within Image J (NIH) was used to measure DAB/Hematoxilin ratio within the region. Three to five individual fields were measured per tissue and mean values were calculated in Microsoft® Excel for Mac (version 16.15).

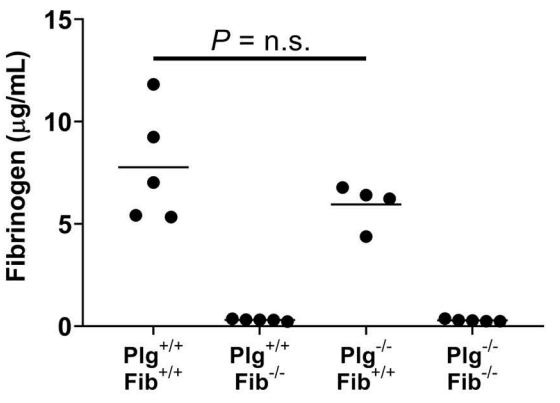
1. Prasad JM, Gorkun OV, Raghu H, et al. Mice expressing a mutant form of fibrinogen that cannot support fibrin formation exhibit compromised antimicrobial host defense. *Blood*. 2015;126(17):2047-2058.
2. Ryu JK, Petersen MA, Murray SG, et al. Blood coagulation protein fibrinogen promotes autoimmunity and demyelination via chemokine release and antigen presentation. *Nat Commun*. 2015;6:8164.

Supplemental Figure Legends

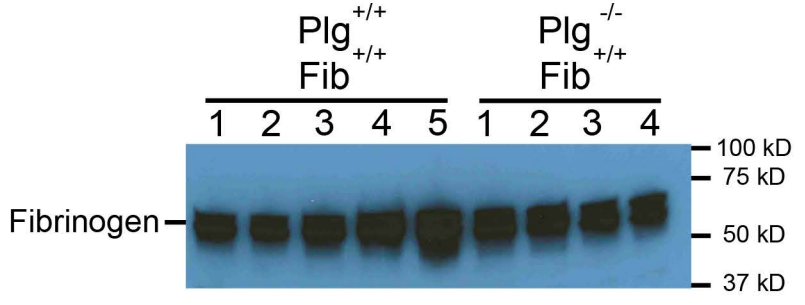
Supplemental Figure 1. **Diminished high-molecular weight fibrin species in Plg^- animals following thioglycollate-induced peritonitis.** (A) Fibrinogen ELISA reveals similar levels of fibrinogen in the peritoneal lavage fluid of both Plg^+ and Plg^- animals after thioglycollate-induced peritonitis. (B) Western blot analysis, under reducing conditions, of the peritoneal lavage fluid also confirms similar fibrinogen level between Plg^+ and Plg^- mice. (C) Interestingly, increased high-molecular weight fibrin species was observed in the lavage fluid from Plg^+ mice compared to Plg^- animals.

Supplemental Figure 2. **Gene dosage effect on macrophage migration in $Fib\gamma^{390-396A}$ animals.** Total cells (A) and macrophages (B) were assessed following thioglycollate-induced peritonitis in Fib^{WT}, $Fib\gamma^{HET}$, and $Fib\gamma^{390-396A}$ mice. $Fib\gamma^{HET}$ animals demonstrated an intermediate phenotype between the Fib^{WT} and $Fib\gamma^{390-396A}$ mice. Both $Fib\gamma^{HET}$ and $Fib\gamma^{390-396A}$ mice had a statistically significant increase in macrophage migration compared to Fib^{WT} animals.

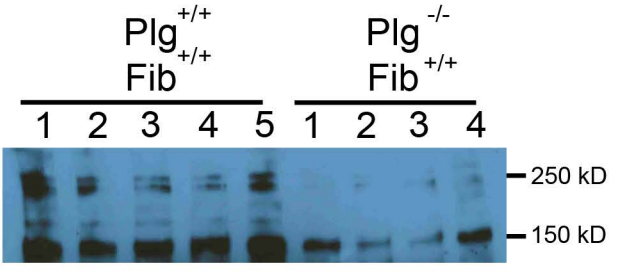
A

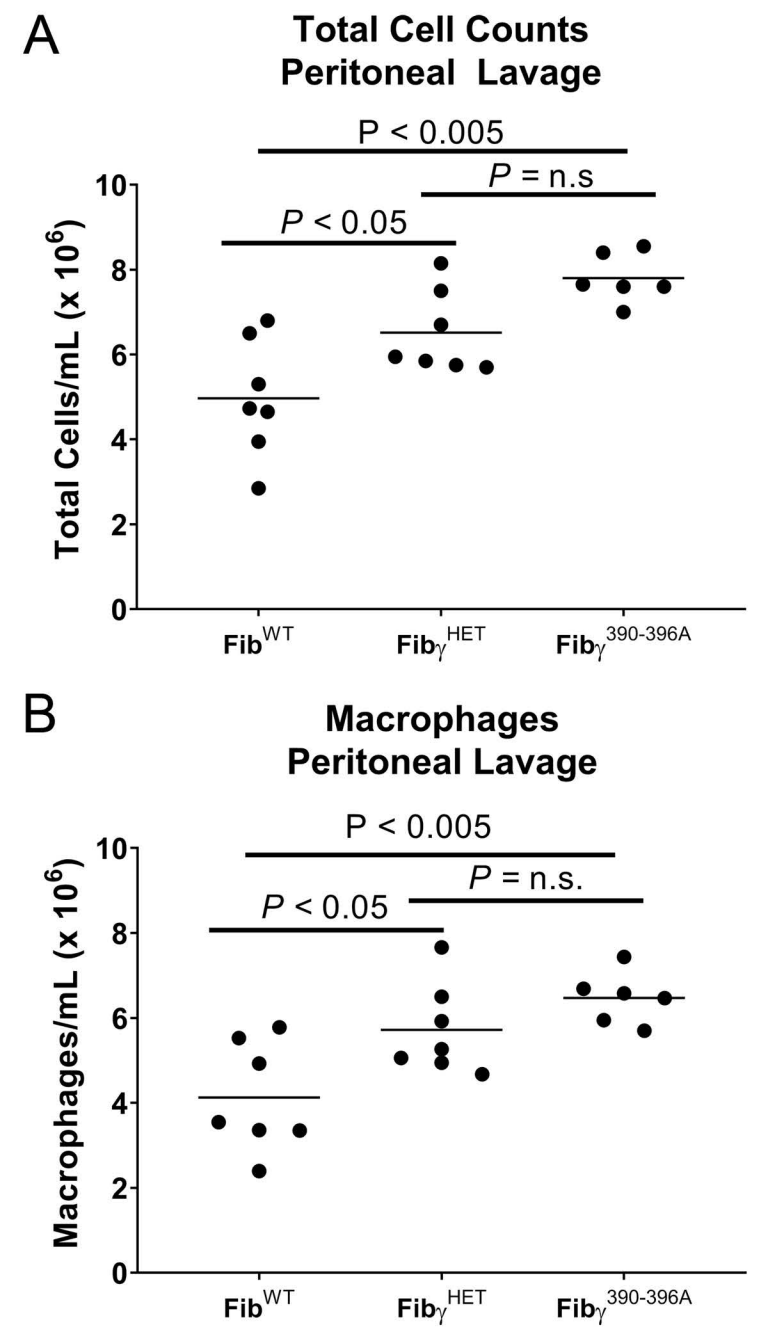

Fibrinogen
Peritoneal Fluid
P = n.s.
Fibrinogen (μg/mL)
15
10
5
0
Plg+/+ Fib+/+
Plg+/+ Fib-/-
Plg-/- Fib+/+
Plg-/- Fib-/-

B


Plg+/+ Fib+/+
Plg-/- Fib+/+
1 2 3 4 5 1 2 3 4
Fibrinogen
100 kD
75 kD
50 kD
37 kD

C


Plg+/+ Fib+/+
Plg-/- Fib+/+
1 2 3 4 5 1 2 3 4
250 kD
150 kD


A
Total Cell Counts
Peritoneal Lavage
P < 0.005
P = n.s
P < 0.05
Total Cells/mL (x 10^6)
10
8
6
4
2
0
Fib^WT
Fibγ^HET
Fibγ^390-396A
B
Macrophages
Peritoneal Lavage
P < 0.005
P = n.s.
P < 0.05
Macrophages/mL (x 10^6)
10
8
6
4
2
0
Fib^WT
Fibγ^HET
Fibγ^390-396A