

SUPPLEMENTAL MATERIAL

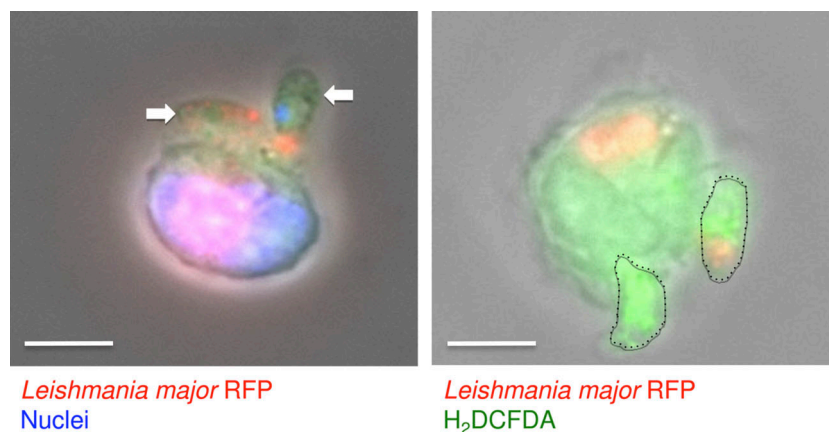
Goncalves et al., <http://www.jem.org/cgi/content/full/jem.20101751/DC1>

Figure S1. ROS production by GR1⁺ monocytes. Blood monocytes (F4/80⁺/CX3CR1^{low}/GFP^{low}/GR1⁺) were sorted by flow cytometry as described in Materials and methods, and incubated with *L. major*-RFP parasites for 30 min. H₂DCFDA was then added for an additional 30 min. ROS production as indicated by H₂DCFDA (green) was monitored by a fluorescent microscopy. Fluorescent and phase-contrast microscopy of a single monocyte with two *L. major* parasites peripherally attached (left panel, white arrows). The monocyte was counterstained by Hoechst 3342 (blue). The image on the right panel shows a single monocyte with two adherent *L. major* parasites (dashed circles) and strongly producing ROS as indicated by H₂DCFDA (green). These pictures are representative of many cells observed in three independent experiments, as described in Fig. 5 E. Bar, 5 μ m.

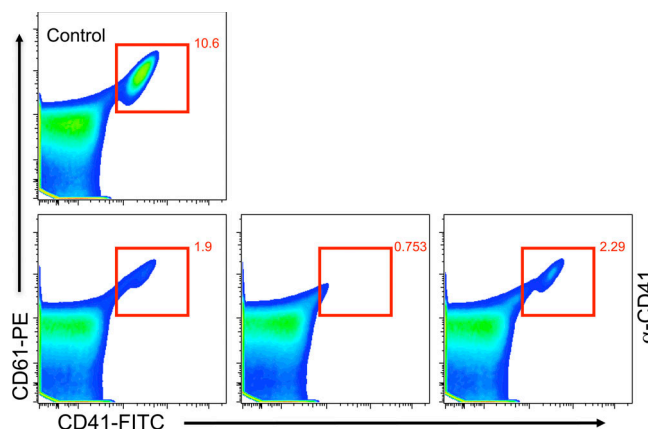


Figure S2. Depletion of mouse platelets by intraperitoneal injection of α -CD41 antibody in vivo. Intraperitoneal injection of α -CD41 antibody to deplete platelets was performed as described in Materials and methods. Flow cytometry analysis of whole blood shows the extent of platelet depletion. Platelets were stained with α -CD61-PE and α -CD41-FITC. Platelet numbers in three mice receiving α -CD41 (bottom row) were variably decreased after treatment with α -CD41 antibody. Platelets from the single mouse treated with control antibody are shown on the top row (10.6%). This experiment was performed twice on a total of six mice.

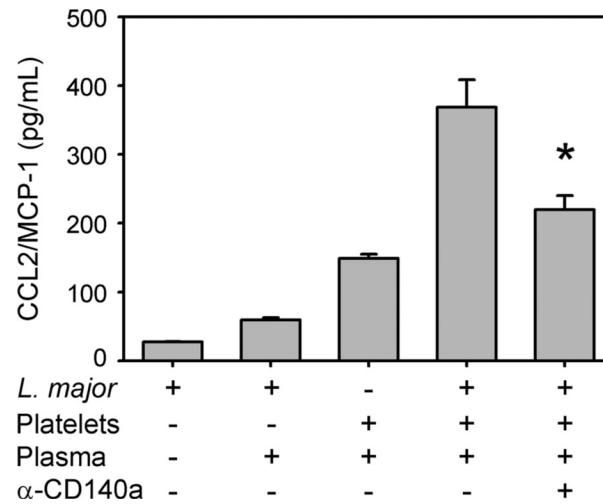


Figure S3. CCL2 (MCP-1) production from MEFs was inhibited by blocking the PDGF receptor. Platelets were isolated from wild-type mice and incubated with *L. major* promastigotes and MEFs in the presence or absence of plasma as source of complement. The PDGF receptor was blocked with α-CD140a antibody. CCL2 (MCP-1) production was measured by ELISA. *, $P < 0.05$. Data are representative of three individual experiments.

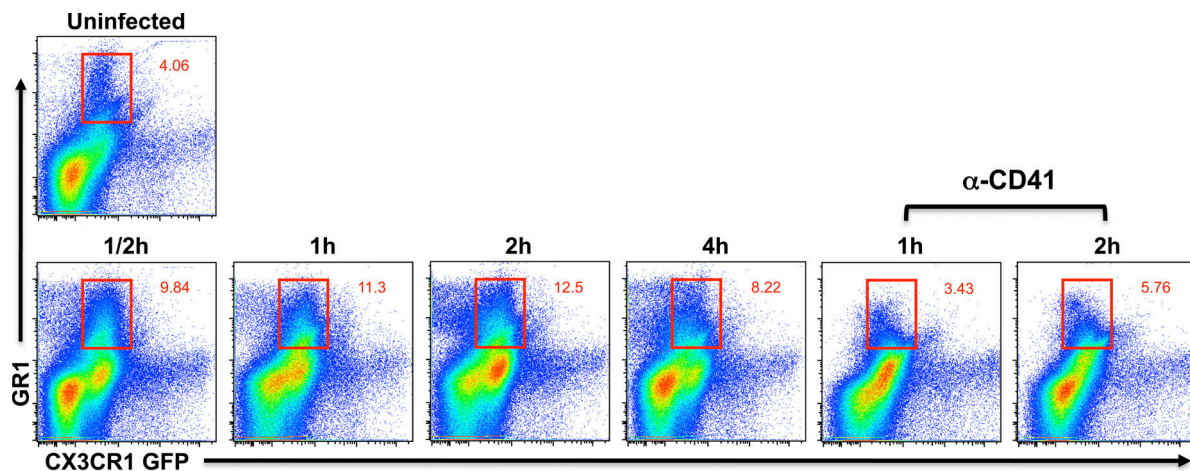
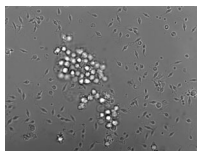
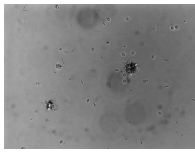


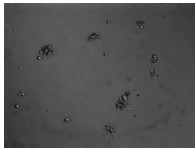
Figure S4. The recruitment of effector monocytes after *L. major* infection in the footpad after platelet depletion. Flow cytometry analyses of the kinetics of the monocyte recruitment into the infected footpads of CX3CR1^{GFP/+}RAG2^{-/-} mice after a total of 4 h infection with 2×10^6 *L. major*. Cells were gated on FSC x SSC profile to eliminate dead cells and debris and stained with F4/80⁺ antibody. The numbers shown in the boxes represent the frequency of gated GR1⁺ cells. This experiment is representative of three individual experiments using three mice per group. The two last dot plots represent a portion of the data shown in Fig. 6 F.



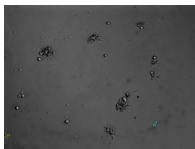
Video 1. In vitro killing of *L. major* parasites by GR1⁺ monocytes. GR1⁺ monocytes were sorted from CX3CR1^{GFP/+}RAG2^{-/-} mice and plated on culture dishes. *L. major* parasites were added in the presence of 5% C5-deficient serum. Parasites were visualized on an inverted microscope 1 h later. Playback speed is 10x and the magnification is 400x. This movie is representative of 2 independent experiments using a total of 20 mice.



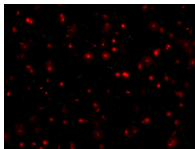
Video 2. In vitro killing of *L. major* parasites by GR1⁻ monocytes. GR1⁻ monocytes were sorted from CX3CR1^{GFP/+} RAG2^{-/-} mice and plated on culture dishes. *L. major* parasites were added in the presence of 5% C5-deficient serum. Parasites were visualized on an inverted microscope 3 h later. Playback speed is 10x and the magnification is 400x. This movie is representative of 2 independent experiments using a total of 20 mice.



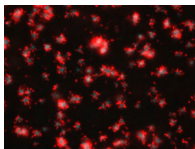
Video 3. Defective killing of *L. major* parasites by GR1⁺ monocytes from Phox^{-/-} mice. GR1⁺ monocytes were sorted from PBMCs from Phox^{-/-} mice based on F4/80 and GR1 expression. *L. major* parasites were added in the presence of 5% C5-deficient serum. Parasites were visualized on an inverted microscope 3 h later. Playback speed is 10x and the magnification is 400x. This movie is representative of 2 independent experiments using a total of 20 mice.



Video 4. The tracking of viable *L. major* parasites after their incubation with GR1⁺ monocytes from Phox^{-/-} mice. GR1⁺ monocytes and *L. major* parasites were incubated as described in Video 5, and parasite motility was tracked with NIH ImageJ software. Playback speed is 10x and the magnification is 400x.



Video 5. *Leishmania* and platelets in C3-deficient serum. Time-lapsed fluorescent microscopy of CFP-platelets and RFP-*L. major* after the addition of 5% serum from a mouse genetically deficient in the third component of complement (C3^{-/-}). Playback speed is 300x and the magnification is 200x. Total incubation time was 20 min. This movie is representative of at least 10 independent experiments.



Video 6. *Leishmania* and platelets in mouse serum containing opsonic complement. Time-lapsed fluorescent microscopy of CFP-platelets clumping around RFP-*L. major* after the addition of 5% serum from a C5-deficient mouse. Playback speed is 300x and the magnification is 200x. Total incubation time was 20 min. This movie is representative of at least 10 independent experiments.