Supporting Information

Sun et al. 10.1073/pnas.1206573109

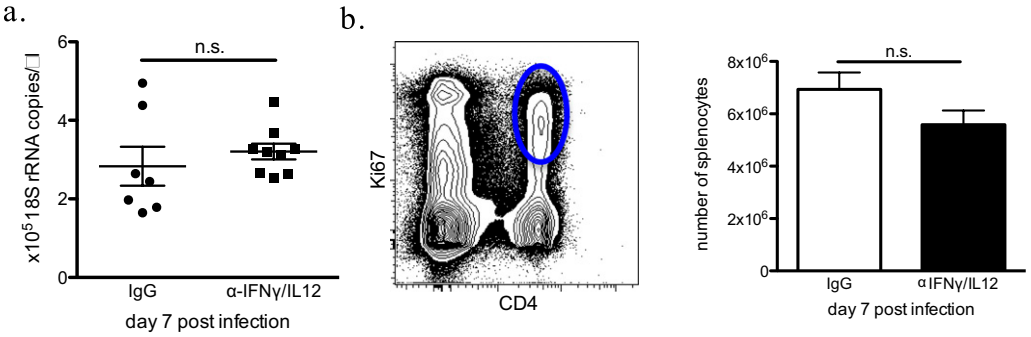

Fig. S1. Parasitemia and CD4 T-cell responses in anti–IFN-γ/IL-12– and control IgG-treated mice. (*A*) Control IgG- and anti–IFN-γ/IL-12–treated mice had comparable parasitemias as detected by quantitative PCR for *Plasmodium berghei* 18S rRNA copies. $n = 7–9$ animals per group. Data are shown as mean ± SEM. (*B*) Proliferation was used as a surrogate for *P. berghei*-responsive CD4 T cells. *P, berghei* ANKA (PbA)-responsive CD4 T cells were identified as $Ki67^+$, $CD4^+$ cells (*Left*). The control IgG- and αIFN-γ/IL-12–treated mice had comparable numbers of PbA-responsive CD4 T cells (*Right*). Data are representative of two independent experiments and are shown as mean ± SEM. $n = 4–5$ animals per group.

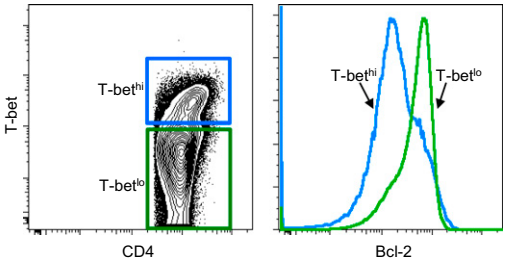

Fig. S2. Bcl-2 expression by CD4 T cells. The level of Bcl-2 was decreased in splenic CD4 T cells that express high versus low levels of T-bet on day 5 post PbA infection, as determined by flow cytometry.

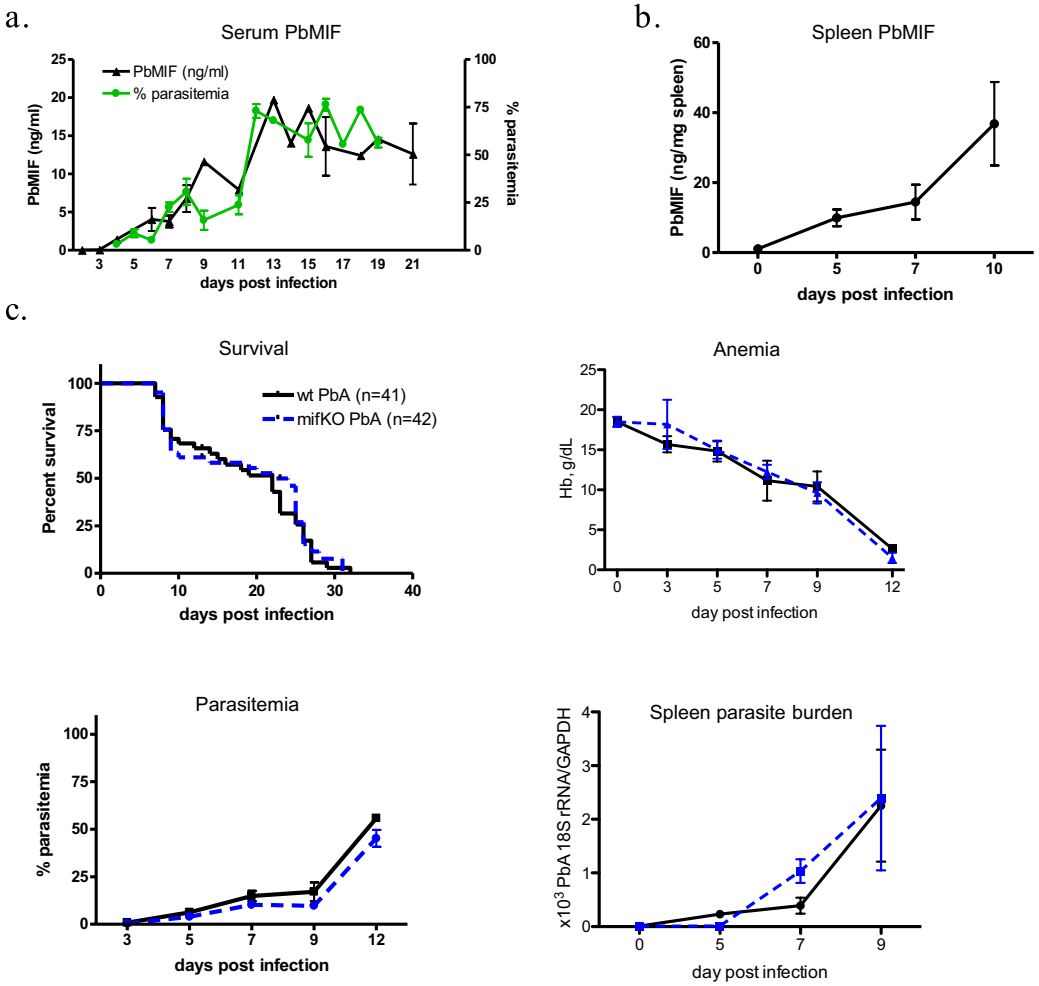

Fig. S3. Detection of *Plasmodium*-encoded macrophage migration inhibitory factor (PMIF) and clinical course of WT PbA and PbA with a genetic deletion in *P. berghei* macrophage migration inhibitory factor (mifKO PbA) infection. (*A*) Serum levels of PbMIF were measured by ELISA (black) and plotted with parasitemia (green), which was determined by counting at least 400 red blood cells (RBCs) on a thin blood smear. $n = 4$ mice per group. (*B*) PbMIF also was detected in spleen lysates by ELISA. $n = 4$ mice per group. (*C*) (*Upper Left*) Survival of BALB/c mice after i.p. injection of 10^6 WT PbA- or mifKO PbA-infected RBCs, $n = 41$ or 42 mice. (*Upper Right*) Anemia was assessed by measuring hemoglobin levels of mice infected with blood-stage WT PbA or mifKO PbA. At the indicated days post infection, 5 μL tail vein blood was collected and diluted in Drabkin's solution, and optical density was determined. Data are representative of three independent experiments. $n = 4$ mice per group. (*Lower Left*) Peripheral blood parasitemia was measured by counting at least 400 RBCs on a thin blood smear to determine numbers of infected RBCs relative to total RBCs. (*Lower Right*) Spleen parasite burden was determined by real-time PCR analysis of the expression of PbA 18S rRNA relative to mouse GAPDH in spleen homogenates at indicated days post infection. Data are shown as mean ± SEM and are representative of in three independent experiments for anemia and blood and spleen parasitemia measurements. $n = 4$ mice per group.

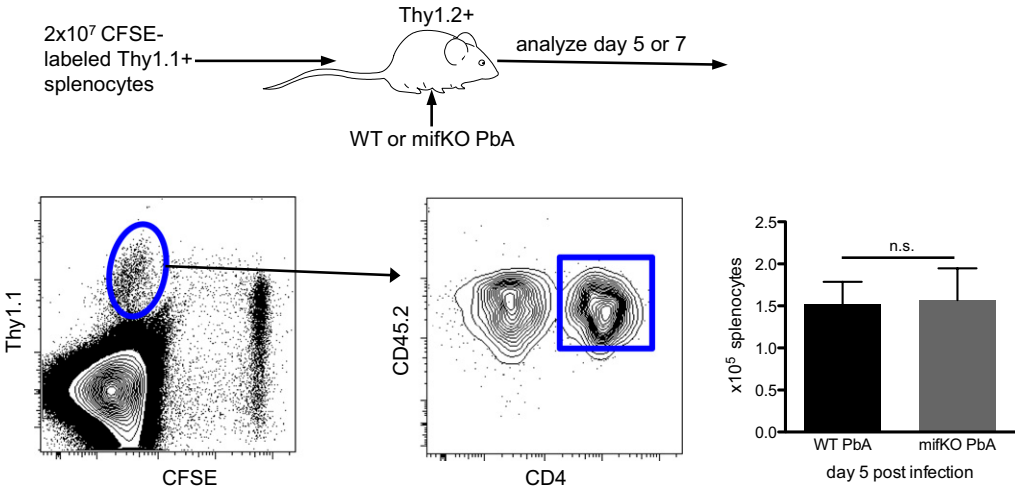

Fig. S4. Detecting PbA-responsive CD4 T cells during WT PbA or miKO PbA infection. Thy1.1$^+$ BALB/c splenocytes (2×10^7) were labeled with 5 μM carboxyfluorescein succinimidyl ester (CFSE) and transferred i.v. on the day of infection. PbA-responsive CD4 T cells were identified as CFSE^{lo}, Thy1.1$^+$, CD4$^+$ cells in intracellular cytokine-staining experiments during which splenocytes were cocultured ex vivo with antigen-presenting cells (APCs) and infected RBC lysates. The PbA-responsive CD4 T cells from the infected host were further identified as CD45.2$^+$ (*Left*). At day 5 post infection, WT PbA- and mifKO PbA-infected mice had similar numbers of PbA-responsive CD4 T cells, identified here as CFSE^{lo}, Thy1.1$^+$, CD4$^+$ splenocytes (*Right*). Data are shown as mean ± SEM and are representative of six independent experiments. n = 3–5 mice per group.

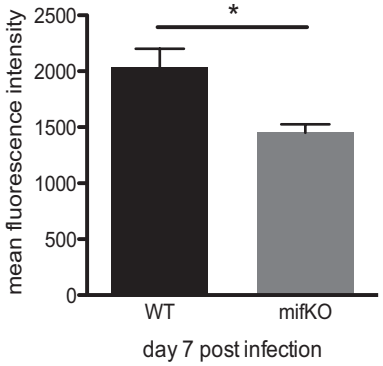

Fig. S5. Production of IFN-γ by CD4 T cells is increased in the presence of PMIF. IFN-γ production by malaria-responding CD4 T cells (CFSE^{lo}, Thy1.1$^+$, CD4$^+$) was increased at day 7 post infection in WT PbA- compared with mifKO PbA-infected mice, shown as mean fluorescence intensity. Data are representative of three independent experiments. n = 4 mice per group. *$P < 0.05$ by two-tailed t test.

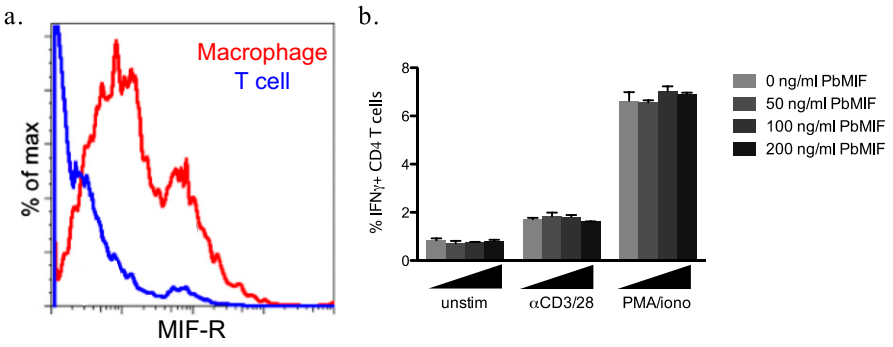

Fig. S6. PMIF signals through the host macrophage migration inhibitory factor receptor (MIF-R) on activated antigen presenting cells (APCs). (*A*) Expression of the MIF-R (also known as "CD74") on macrophages (F4/80$^+$) compared with CD4$^+$ T cells isolated from the spleen of naive BALB/c mice. (*B*) CD4 T cells from naive BALB/c mice were isolated by magnetic separation, and 10^6 CD4 T cells were cultured with increasing concentrations of PbMIF and no other stimulation (unstim), with a 1:1 ratio of anti-CD3/28 beads for 18 h (αCD3/28), or with 50 ng/mL phorbol 12-myristate 13-acetate and 1 μg/mL ionomycin (PMA/iono) for 5 h. Brefeldin A (1 μg/mL) was added to all conditions for the last 3 h, and IFN-γ production was detected by intracellular cytokine staining. Data are shown as mean ± SEM.

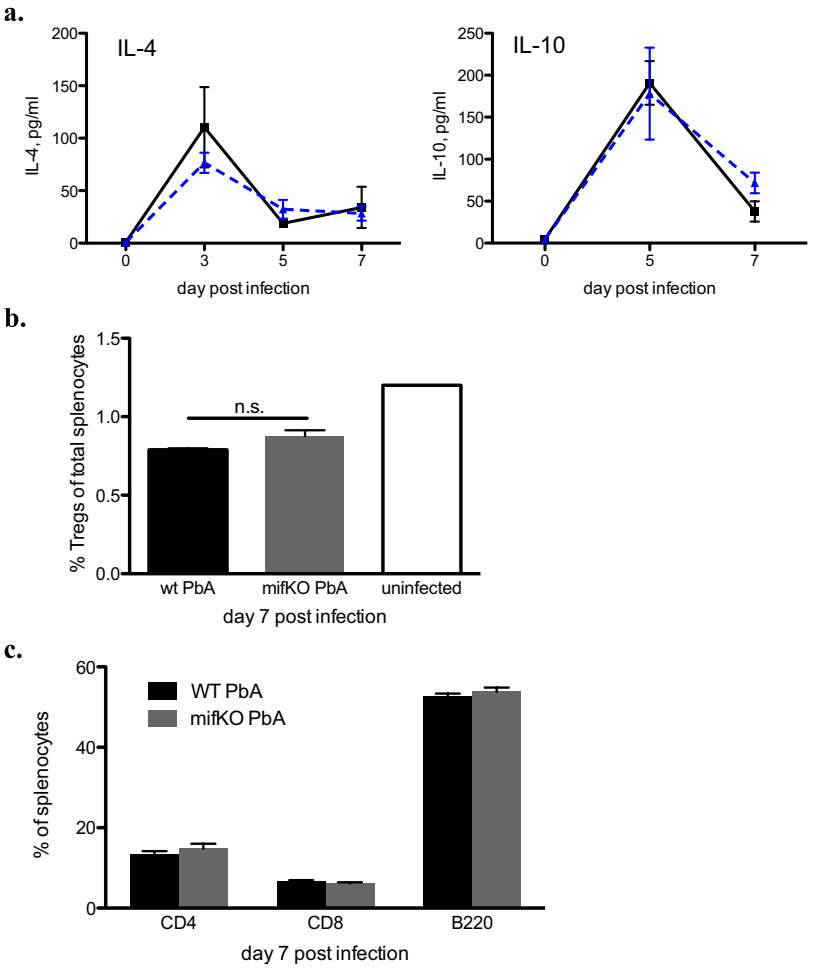

Fig. S7. Presence of PMIF does not alter noninflammatory responses or cell composition. (*A*) Serum levels of the indicated cytokines detected in BALB/c mice infected with WT (solid line) or mifKO (dashed line) PbA, Data are representative of four independent experiments. $n = 3–5$ per group. (*B*) Percent of regulatory T cells (Tregs; $CD4^+$, $CD25^+$, $Foxp3^+$) in WT PbA- and mifKO PbA-infected mice at day 7 post infection. (*C*) Splenic proportions of CD4 T cells ($CD3^+$, $CD4^+$), CD8 T cells ($CD3^+$, $CD8^+$), and B cells ($B220^+$) in WT PbA- and mifKO PbA-infected mice on day 7 post infection. Data are shown as mean ± SEM.

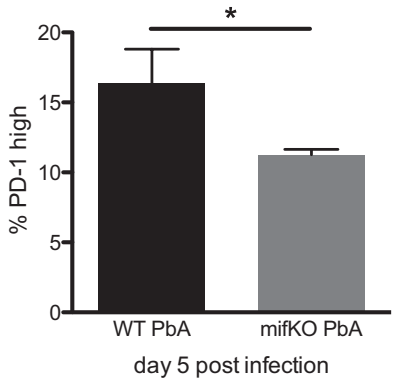

Fig. S8. Presence of PMIF promotes an exhausted CD4 T-cell phenotype. PD-1 levels were assessed in PbA-responsive CD4 T cells ($Ki67^+$ $CD4^+$). A greater percentage of CD4 T cells expressing high levels of PD-1 was observed in WT PbA- than in mifKO PbA-infected mice on day 5 post infection. Data are shown as mean ± SEM. *$P < 0.05$ by two-tailed *t* test.

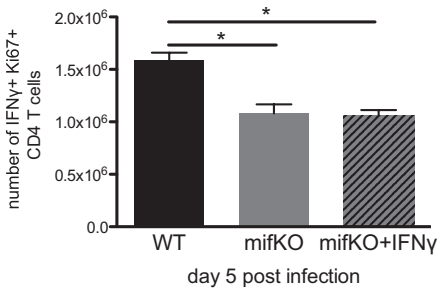

Fig. S9. Administration of IFN-γ does not alter IFN-γ production by responding CD4 T cells. Ten micrograms IFN-γ or PBS were injected i.p. on days 0, 2, and 4 post infection. Mice were killed on day 5 post infection, and intracellular cytokine staining for IFN-γ was performed after 3 h of ex vivo stimulation with PMA and ionomycin in the presence of Brefeldin A. Numbers of IFN-γ–producing $Ki67^+$ $CD4^+$ (PbA-responsive) splenocytes were determined. Data are shown as mean ± SEM and are representative of two independent experiments. $^*P < 0.05$ by two-tailed *t* test. $n = 4$ animals per group.