Supplementary data

CD47-SIRPα Interactions Regulate Macrophage Uptake of *Plasmodium falciparum*-infected Erythrocytes and Clearance of Malaria in vivo.

Kodjo Ayi,[a] Ziyue Lu,[a] Lena Serghides,[a] Jenny M. Ho,[b] Jean CY Wang,[b] W. Conrad Liles,[a,c,d,e] and Kevin C. Kain[a,c,e]*

SAR Laboratories, Sandra Rotman Centre for Global Health, Toronto General Hospital-University Health Network, University of Toronto, Ontario, Canada[a], Princess Margaret Cancer Centre and Department of Medicine, University of Toronto, Ontario, Canada[b], Institute of Medical Science, University of Toronto, Ontario, Canada[c], Department of Medicine, University of Washington, Seattle, Washington, USA[d], Tropical Disease Unit, Division of Infectious Diseases, Department of Medicine, Toronto General Hospital-University Health Network, University of Toronto, Ontario, Canada[e].

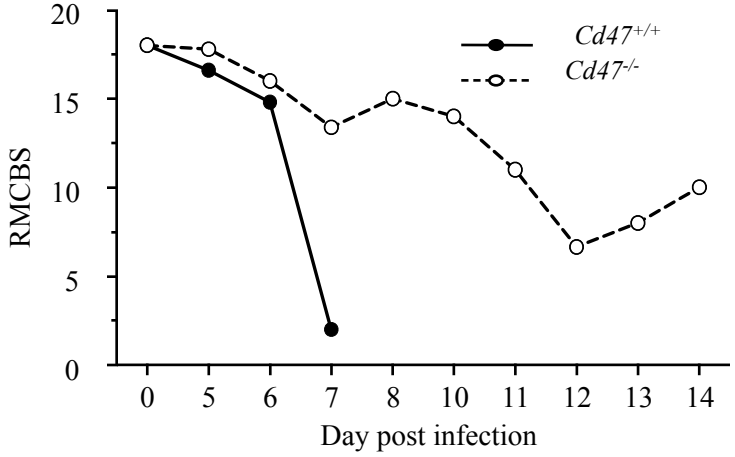

FIG. S1. Rapid murine Coma and behavior scale for quantitative assessment of murine cerebral malaria (RMCBS) in *Cd47*$^{+/+}$ and *Cd47*$^{-/-}$ mice infected with *Plasmodium berghei* ANKA as described (1). Mean RMCBS for *Cd47*$^{+/+}$ mice (solid black line, 2 ± 1, n = 5) and *Cd47*$^{-/-}$ mice (dashed black line, 13.4 ± 4.7, n = 5). Both lines are on a C57BL/6 background, which is susceptible to experimental cerebral malarial (ECM). Slopes of mean RMCBS curves are significantly different between infected *Cd47*$^{+/+}$ mice compared to infected *Cd47*$^{-/-}$ mice ($P < 0.0001$, Two-way ANOVA). The difference in RMCBS at day 7 of infection was also significantly different ($P = 0.011$, Mann Whitney). These data indicate that *Cd47*$^{-/-}$ mice are less susceptible to ECM and neurological injury.

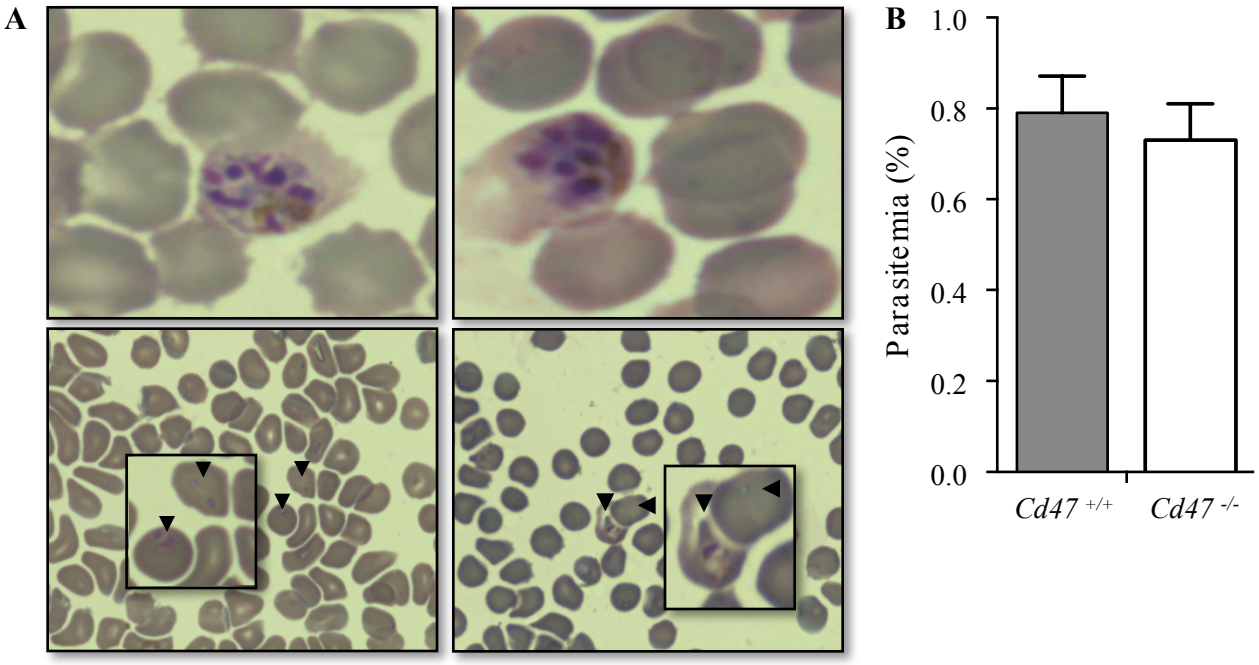

FIG. S2. Reinvasion of *Cd47*$^{+/+}$ RBCs and *Cd47*$^{-/-}$ RBCs with *Plasmodium berghei* ANKA in vitro. Infected mature-stage RBCs where fractioned on Percoll gradient and co-incubated with *Cd47*$^{+/+}$ RBCs (A, left panel) and *Cd47*$^{-/-}$ RBCs (A, right panel) at hematocrit 5% and 0.5% parasitemia in 50% RPMI-1640 and 50% heat-inactivated foetal bovine serum supplemented with, 4 ml of 1 M HEPES, 0.5 mg hypoxanthine, 0.2 g glucose, 0. 4 ml gentamycin, 1 ml of 200 mM glutamine and 2 ml of 5% $NaHCO_3$. Cultures were incubated in T25 tissue-culture flask for 24 h at 37°C in 1% Oxygen, 3% CO_2 and 95% Nitrogen mix gas. Each day the cultures were spun down and fresh growth media was added as described (2). Smears were prepared and stained with diff Quick. The arrowheads show ring-stage parasites. (B) The bar graphs represent the parasitemia levels of *Cd47*$^{+/+}$ RBCs (grey bar) and *Cd47*$^{-/-}$ RBCs (white bar).

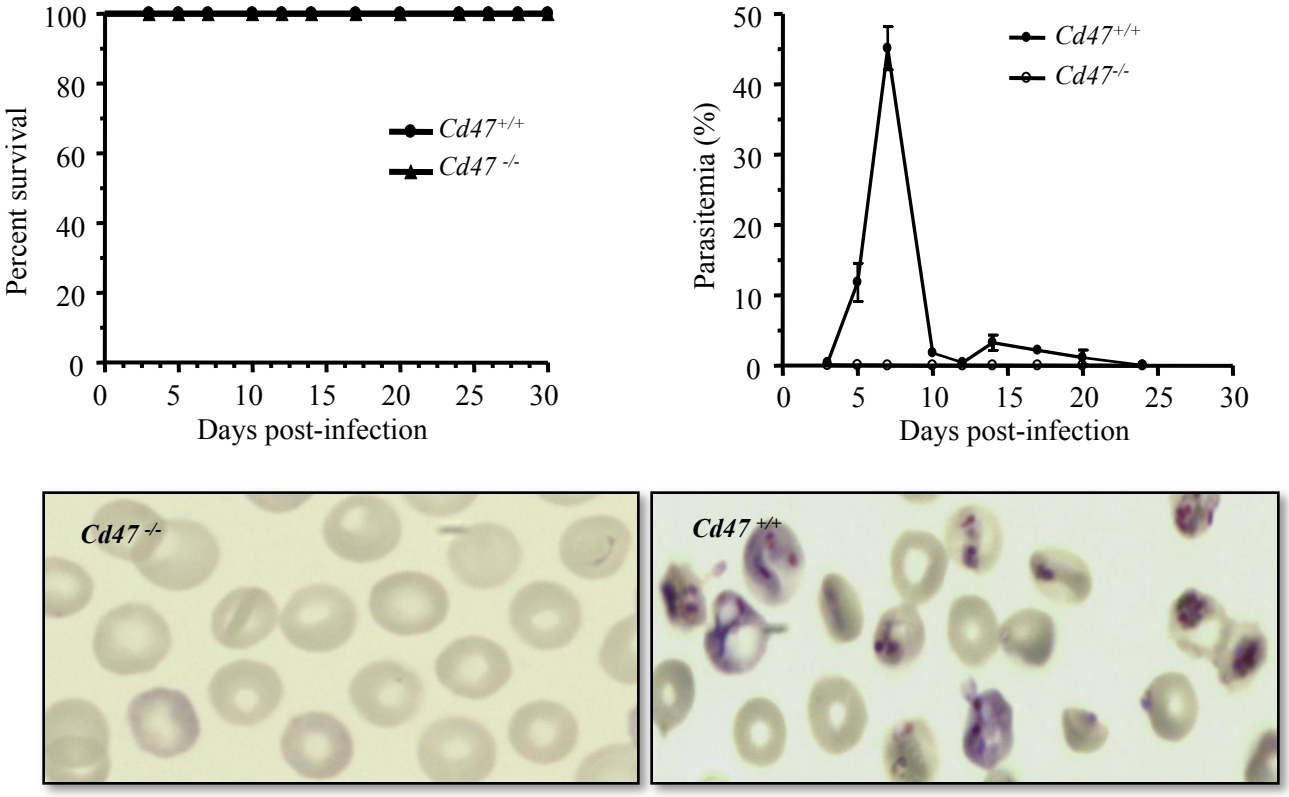

FIG. S3. *Cd47*$^{-/-}$ mice are refractory to *P. chabaudi chabaudi* AS *(*PccAS*)* infection. *Cd47*$^{+/+}$ and *Cd47*$^{-/-}$ 6 to 10-week old mice were injected with 10^6 infected PccAS RBCs. Micrographs show smears at day 7 post infection. (Data presented at ASTMH, 2013, 62 annual meeting, Ayi K. et al).

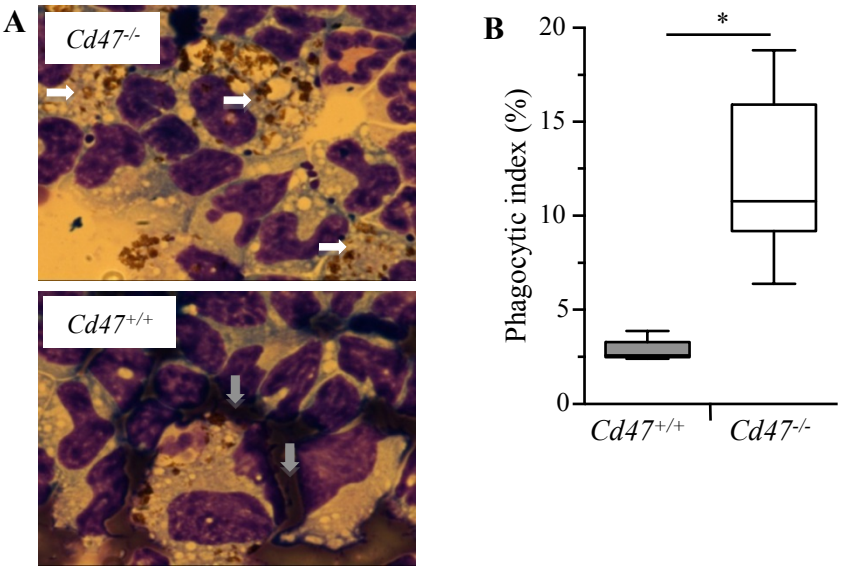

FIG S4 In vivo phagocytosis of ItG mature-stage parasitized erythrocytes (mRBCs) in *Cd47*$^{-/-}$ mice versus *Cd47*$^{+/+}$ mice. CD47-SIRPα disruption activates macrophages and enhances phagocytic activity. 50x10^{6} infected mRBCs, or uninfected RBCs, were injected into the peritoneal cavity of *Cd47*$^{-/-}$ or *Cd47*$^{+/+}$ mice. Twenty hours after injection, peritoneal macrophages were collected, and washed with RPMI-1640 media. 50 µl of the suspension were cytospun at 800 RPM for 10 minutes and stained with Diff-quick. Images from the slides were acquired with an Olympus BX41 microscope and an Infinity2 camera at 1000x magnification. (A) Shows representative image of light microscopy pictures of peritoneal macrophages with internalized mRBCs erythrocytes (white arrows) and non-internalized mRBC (grey arrows). Images from the slides were acquired with an Olympus BX41 microscope and an Infinity2 camera at 1000x magnification. (B) Phagocytosis was assessed as the percentage of monocytes with at least 1 internalized mRBC. Data were analyzed by the Mann Whitney test and are the combined results of three experiments (*, $P < 0.0001$).

REFERENCES

1. **Carroll RW, Wainwright MS, Kim KY, Kidambi T, Gómez ND, Taylor T, Haldar K.** 2010. A rapid murine coma and behavior scale for quantitative assessment of murine cerebral malaria. PLoS One. **5**:e13124.
2. **McNally J. O'Donovan SM, Dalton JP.** 1992. *Plasmodium berghei* and *Plasmodium chabaudi chabaudi*: development of simple in vitro erythrocyte invasion assays. Parasitology. **105**: 355-62.