

Supplemental Data

Supplemental Figure 1: Relationship between parasite biomass and blood smear and plasma cytokine levels.

Supplemental Figure 2: Proportions of circulating monocyte subsets in children with asymptomatic *P. falciparum* infection are similar to those in uninfected (Pf PCR negative) children.

Supplemental Figure 3: Children with asymptomatic *P. falciparum* infection have altered monocyte surface phenotypes compared to uninfected (Pf PCR negative) children.

Supplemental Figure 4: Controls used for monocyte phagocytosis assays.

Supplemental Figure 5: TLR responsiveness in recovery monocytes does not differ between Pf-uninfected children and those with asymptomatic Pf infection.

Supplemental Figure 6: Cryopreservation does not significantly affect proportions of monocyte subsets.

Supplemental Figure 7: Greater magnitude of phagocytosis of Ghana14 IE compared to 3D7 IE by North American monocytes.

Supplemental Figure 8: Cryopreservation does not affect monocyte inflammatory gene expression profiles.

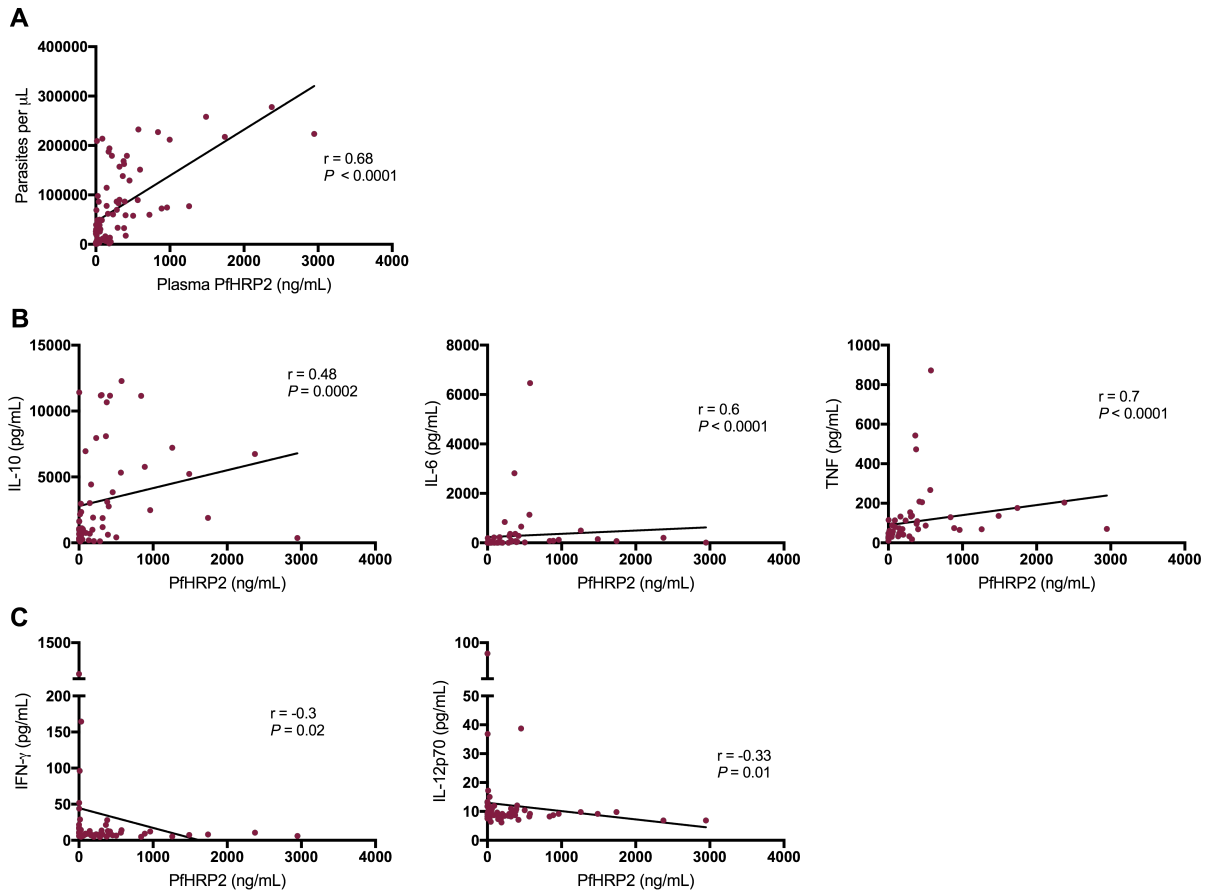
Supplemental Table 1: Clinical presentations and laboratory features of the study population.

Supplemental Table 2: Acute uncomplicated malaria is associated with a robust pro-inflammatory cytokine response and systemic inflammation.

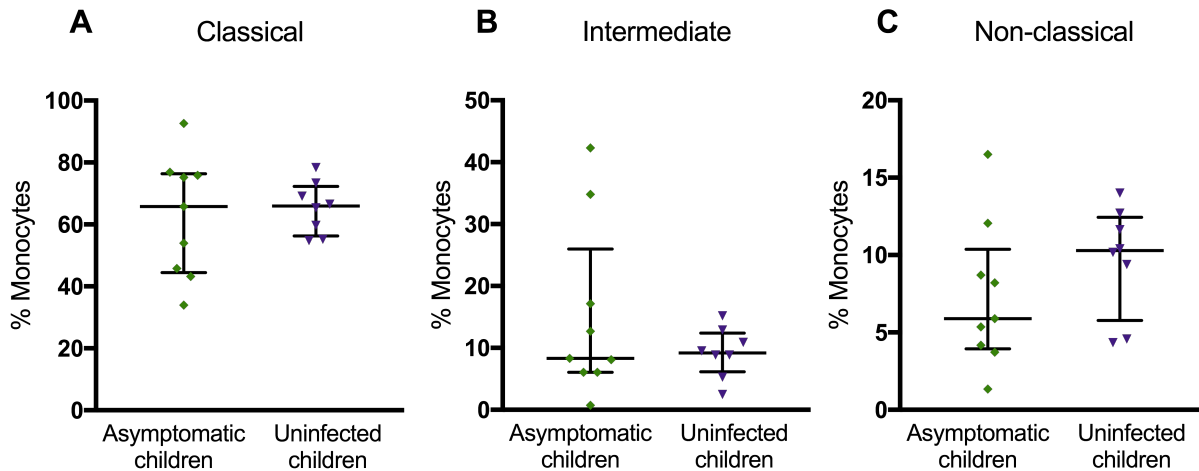
Supplemental Table 3: Children with asymptomatic parasitemia have elevated inflammatory cytokines compared to uninfected children.

Supplemental Table 4: Pathway enrichment during acute malaria compared to recovery.

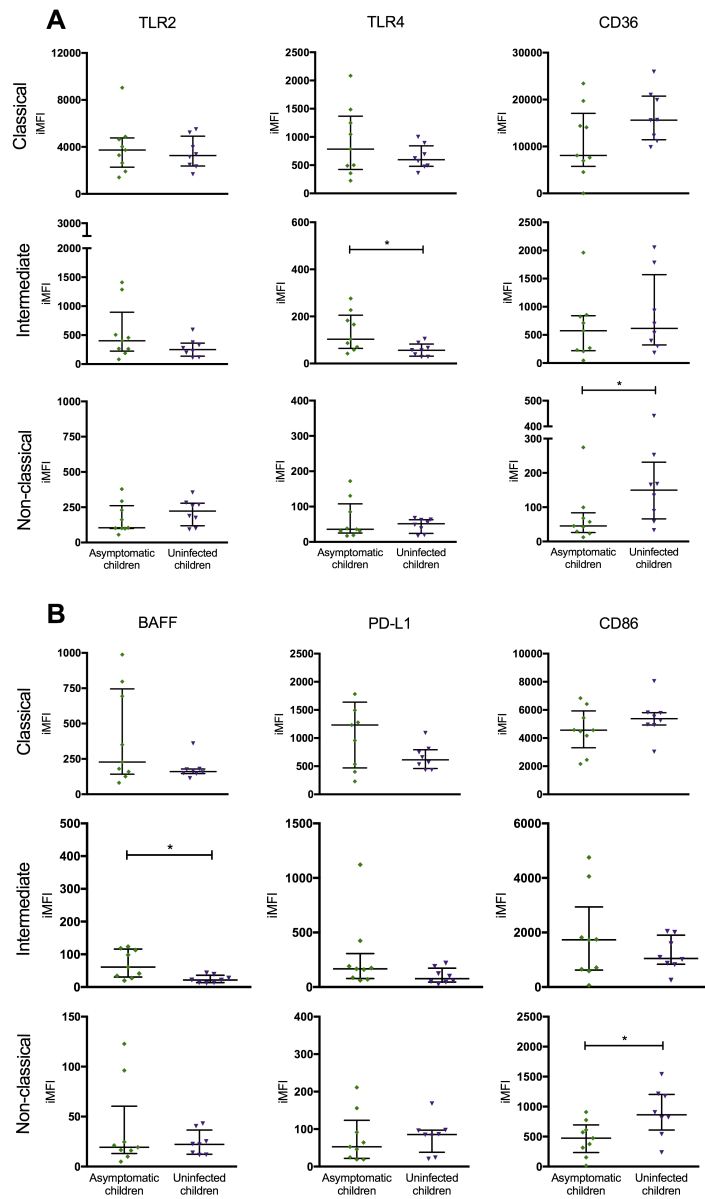
Supplemental Data File 1: Monocyte inflammatory gene expression data.



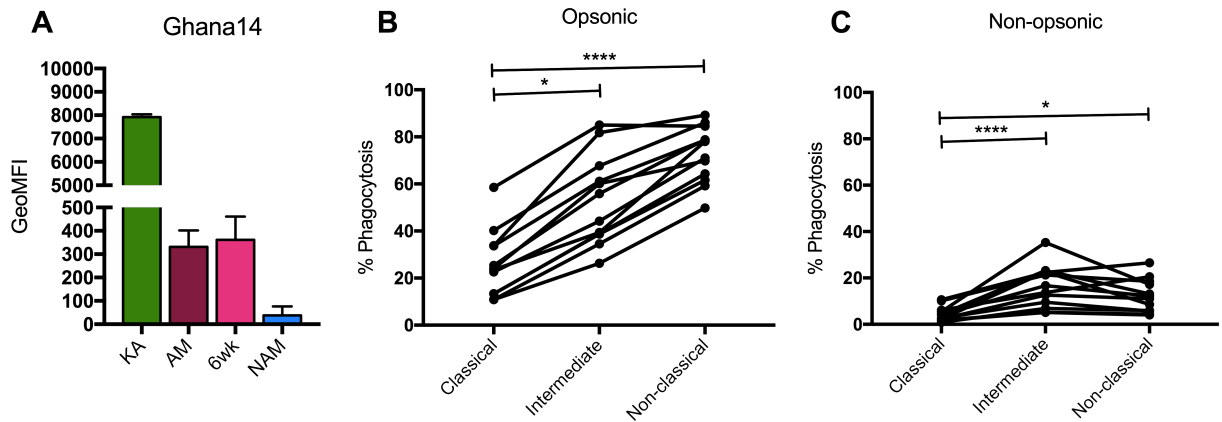
Supplemental Figure 1. Relationship between plasma PfHRP2 and blood smear and plasma cytokine levels. (A) PfHRP2 directly correlated with blood smear results ($n = 79$). **(B)** Plasma PfHRP2 directly correlated with plasma levels of IL-10, IL-6, and TNF. **(C)** Plasma PfHRP2 inversely correlated with plasma levels of IFN- γ and IL-12p70 ($n = 56$ cases of uncomplicated malaria with corresponding PfHRP2 and plasma cytokine data). Spearman correlation was used to determine relationships between the variables. PfHRP2: *P. falciparum* histidine-rich protein 2.



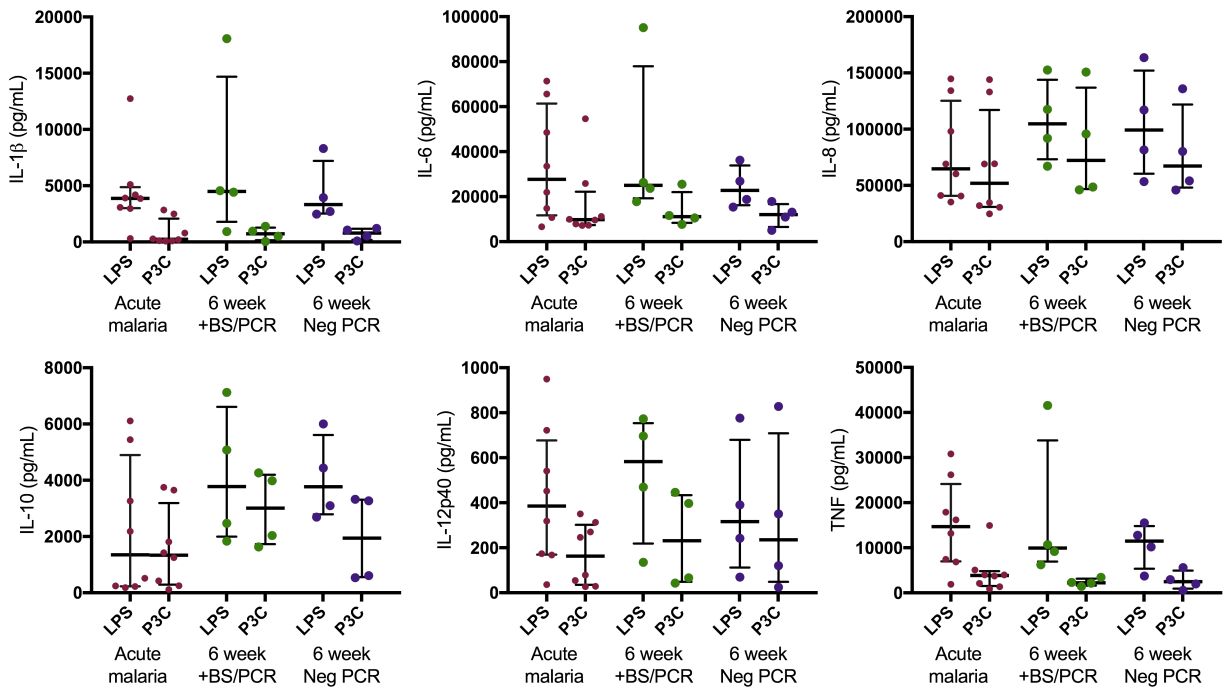
Supplemental Figure 2. Proportions of circulating monocyte subsets in children with asymptomatic *P. falciparum* infection are similar to those of uninfected (Pf PCR negative) children. The 3 subsets were determined based on CD14 and CD16 expression (classical CD14⁺⁺CD16⁻, intermediate CD14⁺⁺CD16⁺, and non-classical CD14⁺CD16⁺⁺). Proportions of all circulating monocytes that are (A) classical, (B) intermediate, and (C) non-classical for healthy child controls with asymptomatic infection determined by blood smear or Pf PCR ($n = 9$) and uninfected (Pf PCR negative) children ($n = 8$). Mann-Whitney test was used to compare the groups. Bars represent median values with interquartile ranges.



Supplemental Figure 3. Children with asymptomatic *P. falciparum* infection have altered monocyte surface phenotypes compared to uninfected (Pf PCR negative) children. Flow cytometry was used to determine cell surface expression of 6 markers on the 3 monocyte subsets (classical, intermediate, and non-classical) for healthy child controls with asymptomatic infection determined by blood smear or Pf PCR ($n = 9$) compared to uninfected (Pf PCR negative) children ($n = 8$). Integrated MFI (iMFI) was calculated for each cell surface marker by multiplying the geometric mean fluorescence by the frequency of the parent population. Cell surface expression markers include those important to (A) innate immunity (TLR2, TLR4, and CD36) and to (B) adaptive immunity (BAFF, PD-L1, and CD86). Mann-Whitney test was used to compare the groups. Bars represent median values with interquartile ranges. iMFI, integrated mean fluorescence intensity; BAFF, B-cell activating factor; PD-L1, programmed death-ligand 1.

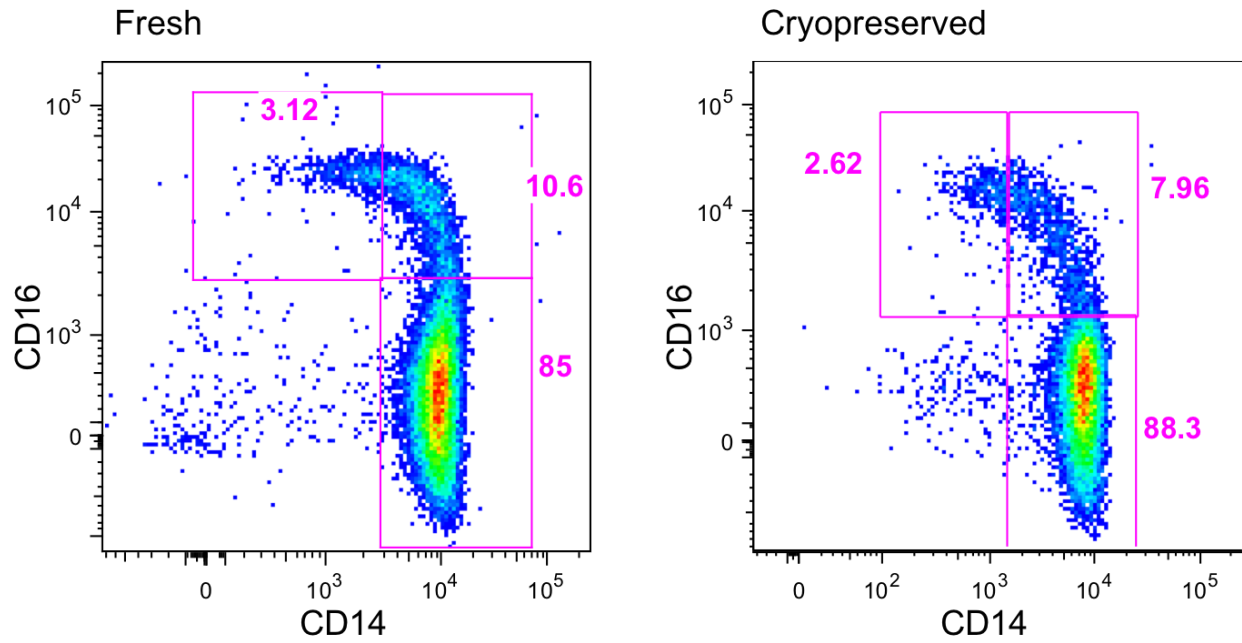


Supplemental Figure 4. Controls used for monocyte phagocytosis assays. (A) Levels of Ghana14 IE variant surface antigen (VSA)-specific IgG in pooled plasma from 8 malaria-immune Kenyan adults (KA) (performed in duplicate), plasma from children with acute malaria (AM) and 6 wks post-treatment ($n = 24$ pairs), and plasma from malaria-naïve North American controls ($n = 2$). Data are shown as means \pm SEM. (B) Opsonic phagocytic function of the three monocyte subsets (classical, intermediate, and non-classical) from a single malaria-naïve North American, repeated in 12 separate experiments on different days, in the presence of Ghana14 IE opsonized with heat-inactivated pooled plasma from Kenyan adults. (C) Non-opsonic phagocytic function of the three monocyte subsets (classical, intermediate, and non-classical) from a single malaria-naïve North American, repeated in 12 separate experiments on different days, in the presence of Ghana14 IE opsonized with heat-inactivated plasma from a malaria-naïve North American. Friedman test was used to compare phagocytic activity among the three monocyte subsets. $*P < 0.05$, $****P < 0.0001$. IE, Pf-infected erythrocytes; GeoMFI, geometric mean fluorescence intensity; KA, pooled plasma from 8 malaria-immune Kenyan adults; AM, acute malaria; 6wk, 6 wk recovery; NAM, North American.

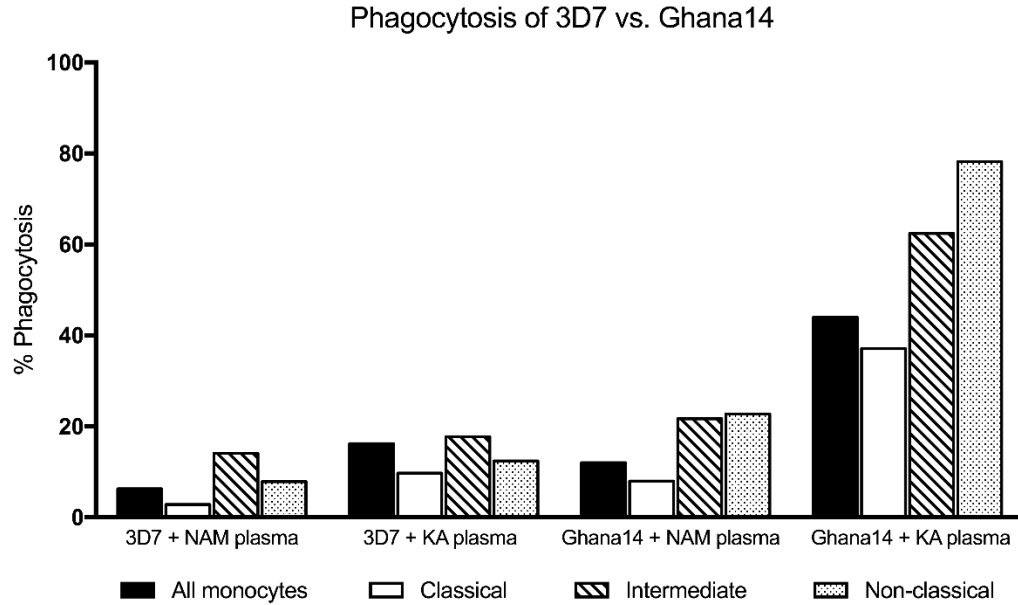


Supplemental Figure 5. TLR4 and TLR2/TLR1 responsiveness in recovery monocytes does not differ between Pf-uninfected children and those with asymptomatic *P. falciparum* infection.

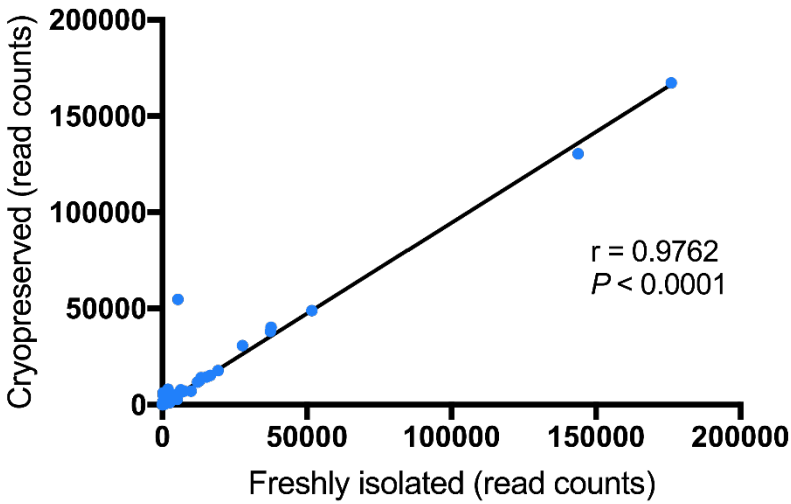
Monocytes were negatively selected from fresh venous blood samples from children during acute malaria and 6 weeks following treatment ($n = 8$ pairs). Half of the children had asymptomatic infection at 6 weeks (6 wk positive blood smear/PCR, $n = 4$) and half were uninfected (6 wk negative PCR, $n = 4$). (North American control data shown in Fig. 7). Cells were cultured for 18 hrs with media alone, LPS 10 ng/mL, or Pam3CSK4 (P3C) 100 ng/mL, and cytokine concentrations were measured in culture supernatants. Kruskal-Wallis test was used to compare acute malaria, 6 wk recovery with asymptomatic Pf infection diagnosed by blood smear or Pf PCR (positive blood smear/PCR), and 6 wk recovery uninfected with Pf (negative PCR). Data are shown as medians with interquartile ranges.



Supplemental Figure 6. Cryopreservation does not significantly affect monocyte subset proportions. Flow cytometry with staining for surface CD14 and CD16 was performed on fresh vs. cryopreserved PBMC from a single North American donor.



Supplemental Figure 7. Greater magnitude of phagocytosis of Ghana14 IE compared to 3D7 IE by North American monocytes, with similar pattern of activity among the monocyte subsets. NAM, malaria-naive North American plasma; KA, pooled plasma from malaria-immune Kenyan adults; IE, Pf-infected erythrocytes. A single North American PBMC donor was used for all phagocytosis conditions.



Supplemental Figure 8. Cryopreservation does not affect monocyte inflammatory gene expression profiles. Targeted digital RNA sequencing was performed on monocytes isolated via negative selection from fresh vs. cryopreserved PBMC from a single North American donor. A customized panel targeted 508 genes important in inflammation and immunity (QIAseq Human Inflammation and Immunity Transcriptome Panel, Qiagen). The outlier with higher transcript levels with cryopreservation (54,753) compared to fresh isolation (5,343) is for the gene *FOS*. Spearman correlation was used to compare expression levels.

	Acute malaria (N = 95)	6 wk recovery (N = 95)	Healthy children (N = 40)	Healthy adults (N = 14)
Age in yrs – median (range)	4.8 (1 – 10.3)		5.7 (1.2 – 10.6)	28 (18.8 – 35.2)
Percent female	43.2		47.5	78.6
Sickle cell trait – no. (%)	7 (7.4)		7 (17.5)	2 (14.3)
Sickle cell disease – no. (%)	1 (1.1)		0	0
Symptomatic: peripheral blood smear, Pf parasites/μL – median (IQR)	48,870 (10,269 – 101,660)			
Asymptomatic: peripheral blood smear positive –no. (%)		16 (16.8)	8 (20)	0
Asymptomatic: peripheral blood smear, Pf parasites/μL – median (IQR)		6,662 (436.5 – 22,829)	924.8 (309 – 4,410)	
Asymptomatic: Pf PCR positive (blood smear negative) – no. (%)		23 (24.2)	13 (32.5)	6 (42.8)
Plasma PfHRP2, ng/mL – median (IQR)	131.3 (17.3 – 381.3)	n.d.	n.d.	n.d.
Total leukocyte count per μL – median (IQR)	9,000 (6,400 – 11,430)	8,300 (6,700 – 10,630)	8,100 (6,600 – 10,750)	6,800 (5,775 – 7,775)
% Monocytes – median (IQR)	5.2 (3.4 – 7.8)	5.9 (4.2 – 7.8)	5.5 (3.8 – 6.3)	5.1 (3.9 – 6.3)
Monocyte count per μL – median (IQR)	500 (300 - 700)	500 (300 - 700)	450 (300 - 575)	300 (300 - 400)
% Granulocytes – median (IQR)	68.9 (56.7 - 78.6)	40.6 (36.2 – 45.8)****	44 (36.3 – 50.1)****	53.2 (46 – 58.6)
Granulocyte count per μL – median (IQR)	6,300 (3,950 – 8,150)	3,300 (2,700 – 4,600)****	3,400 (2,325 – 4,750)****	3,600 (2,925 – 4,750)
% Lymphocytes – median (IQR)	25.5 (17.6 – 36.6)	53 (48.5 – 57.9)****	50.7 (44.9 – 57.2)****	40.2 (37.4 – 49.2)
Lymphocyte count per μL – median (IQR)	2,200 (1,500 – 3,000)	4,600 (3,450 – 5,550)****	4,150 (3,200 – 4,675)****	2,900 (2,375 – 3,400)
Platelet count per μL – median (IQR)	306,000 (257,000 – 365,000)	494,000 (413,500 – 589,500)****	430,500 (377,000 – 496,300)****	353,000 (312,500 – 470,000)
Hemoglobin, g/dL – median (IQR)	10.1 (9 – 10.9)	10.8 (9.6 – 11.5)****	10.6 (9.9 – 11.4)*	12.4 (10.9 – 13.3)
Plasma heme positive (>1.03 μM) – no. (%)	9/26 (34.6)	5/26 (19.2)	1/14 (7.1)	1/14 (7.1)
Plasma heme (of those positive), μM – median (range)	3.18 (1.03 – 12.44)	2.82 (1.61 – 10.35)	2.09	2.39

Supplemental Table 1. Clinical presentations and laboratory features of the study population. The table includes all participants from whom plasma and/or monocyte samples

were analyzed: cases of acute uncomplicated malaria and matching 6 wk recovery samples ($N = 95$ pairs in total; $n = 60$ for plasma cytokine profiles; $n = 35$ for monocyte subset proportions; $n = 23$ for monocyte cell surface phenotypes; $n = 12$ for monocyte phagocytic function; $n = 8$ for monocyte TLR responses; $n = 6$ for monocyte mRNA), healthy Kenyan child controls ($N = 40$ total; $n = 40$ for plasma cytokine profiles; $n = 17$ for monocyte subset proportions and cell surface phenotypes), and healthy Kenyan adult controls ($N = 14$ for monocyte subset proportions). Plasma heme levels were measured for a subset of 26 acute-recovery pairs, 14 healthy child controls, and 14 healthy adult controls. Fisher's exact test was used to compare proportions of sickle cell trait and disease between cases of acute malaria and healthy child controls. For plasma heme and complete blood cell count parameters, Wilcoxon matched-pairs rank test was used to compare acute malaria to matching 6 wk recovery samples, and Kruskal-Wallis test was used to compare unmatched groups. Parameters in bold type showed statistically significant differences between acute malaria compared to 6 wk recovery samples and/or healthy child controls. $*P < 0.05$; $****P < 0.0001$, as compared to cases of acute malaria. PfHRP2, Pf histidine-rich protein 2; IQR, interquartile range; n.d., not done.

Plasma cytokine	Acute malaria (<i>n</i> = 60)	6 wk recovery (<i>n</i> = 60)	Healthy child controls (<i>n</i> = 40)
IP-10 – pg/mL	5264 (269.9-14,579)	560.3 (83.3-5202)****	360.1 (136.3-7633)****
IFN- γ – pg/mL	9.3 (4.5-1067)	6.3 (3.9-799.7)****	6.1 (3.9-26.4)****
IL-6 – pg/mL	18 (1.8-6461)	1.7 (0.8-138.8)****	2.3 (1.1-29.8)****
TNF – pg/mL	65.4 (10.7-872.3)	20.8 (10.2-71.9)****	19 (5.8-96.2)****
sTNF-R1 – pg/mL	1828 (413-12,763)	373.3 (83.2-4537)****	341.8 (156.4-1184)****
sTNF-R2 – pg/mL	48,551 (9987-118,895)	8792 (2690-75,415)****	8489 (624.3-51,516)****
IL-10 – pg/mL	1649 (91-12,285)	32.2 (9.2-6881)****	25.9 (6.7-440.4)****
sBAFF – pg/mL	1418 (896.1-6734)	1048 (563.8-2651)****	1035 (621.7-2149)****
sCD14 – pg/mL	2807 (804.2-4739)	1427 (530.9-3363)****	1276 (501.9-2946)****
CRP – mg/dL	9.6 (0.23-28.2)	0.26 (0.02-22.6)****	0.15 (0.01-18.5)****
IL-4 – pg/mL	7.4 (4.7-70.9)	6.6 (5.1-65.3)	7.8 (5.9-31.3)
IL-7 – pg/mL	0.58 (0.45-1)	0.65 (0.46-1.6)*	0.51 (0.39-1.7)**
IL-9 – pg/mL	1.8 (0.71-32.4)	2.2 (0.98-20)**	2 (1.2-5.2)
IL-12p40 – pg/mL	17.3 (9-129.8)	16.5 (11.1-50.1)	15.2 (9.7-34.9)
IL-12p70 – pg/mL	9.2 (6.2-84.9)	9.6 (6.4-86.1)	8.6 (6.5-23.5)
IL-17 – pg/mL	4.1 (1.7-145.3)	4.5 (2-112.9)	3.4 (1.6-141.5)

Supplemental Table 2. Acute uncomplicated malaria is associated with a robust pro-inflammatory cytokine response and systemic inflammation. Plasma cytokine and acute phase reactant levels were compared between cases of acute uncomplicated malaria and 6 wk recovery samples (*n* = 60 pairs) and healthy child controls (*n* = 40). Values listed are medians with ranges. Wilcoxon matched-pairs rank test was used to compare acute malaria to 6 wk recovery samples. Kruskal-Wallis test was used to healthy child controls to acute malaria and 6 wk recovery samples. Parameters in bold type showed large, statistically significant differences between acute malaria and 6 wk recovery samples and healthy child controls. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001, as compared to acute malaria cases. IP-10, IFN- γ -induced protein 10; sTNF-R1, soluble TNF receptor 1; sTNF-R2, soluble TNF receptor 2; sBAFF, soluble B cell activating factor; CRP, C reactive protein.

Plasma cytokine profiles: healthy child controls			
	Asymptomatic blood smear positive (<i>n</i> = 8)	Asymptomatic Pf PCR positive and blood smear negative (<i>n</i> = 13)	Uninfected blood smear and Pf PCR negative (<i>n</i> = 19)
Peripheral blood smear Pf parasites/ μ L – median (range)	924.8 (51.5 – 8,908)		
IP-10 – pg/mL	530.4 (224.6 – 2,210)	422.8 (189.1 – 7,633)	312.5 (136.3 – 3,832)
IFN- γ – pg/mL	6 (5.1 – 7.2)	6.3 (4.8 – 26.4)	6 (3.9 – 12.6)
IL-6 – pg/mL	2.8 (1.1 – 12.4)	1.8 (1.2 – 7.5)	2.3 (1.1 – 29.8)
TNF – pg/mL	27.1 (16.2 – 96.2)	14.6 (10.5 – 30.8)*	17.1 (5.8 – 58.4)
sTNF-R1 – pg/mL	410.6 (274.5 – 1,184)	393.2 (215.4 – 628.9)	301.6 (156.4 – 1,082)
sTNF-R2 – pg/mL	9,829 (8,249 – 51,516)	9,331 (4,573 – 31,371)	7,626 (624.3 – 21,164)
IL-10 – pg/mL	57.7 (19.6 – 440.4)	27.6 (7.5 – 362.9)	20.9 (6.7 – 36.3)*
sBAFF – pg/mL	1,168 (947.5 – 1,725)	966.8 (621.7 – 2,098)	1,035 (746.9 – 2,149)
sCD14 – pg/mL	1,206 (937.4 – 2,665)	1,603 (501.9 – 2,593)	1,189 (727.1 – 2,946)
CRP – mg/dL	0.86 (0.1 – 12)	0.28 (0.02 – 18.5)	0.08 (0.01 – 1.1)*
IL-4 – pg/mL	7.6 (6.1 – 14.5)	7.8 (5.9 – 18.4)	7.8 (6.2 – 31.3)
IL-7 – pg/mL	0.49 (0.42 – 1.7)	0.49 (0.39 – 0.86)	0.53 (0.4 – 0.65)
IL-9 – pg/mL	2.2 (1.4 – 5.2)	1.8 (1.2 – 2.7)	2 (1.2 – 3.3)
IL-12p40 – pg/mL	18.2 (14.5 – 24.9)	14.5 (11.4 – 34.9)	14.5 (9.7 – 25.9)
IL-12p70 – pg/mL	9.3 (6.5 – 12.4)	9.8 (7.2 – 23.5)	8.5 (6.5 – 14.7)
IL-17 – pg/mL	4.2 (2 – 10.8)	3.4 (1.6 – 141.5)	3.4 (2.4 – 30.5)

Supplemental Table 3. Children with asymptomatic parasitemia have elevated inflammatory markers compared to uninfected children. Plasma cytokine and acute phase reactant profiles were compared between healthy child controls with asymptomatic infection determined by positive blood smear (*n* = 8), asymptomatic infection determined by positive Pf PCR (blood smear negative) (*n* = 13), and uninfected (Pf PCR negative) children (*n* = 19). Values listed are medians with ranges. Kruskal-Wallis test was used to compare the groups. Parameters in bold type showed statistically significant differences between children with asymptomatic infection (blood smear positive) compared to asymptomatic infection by PCR (Pf PCR positive/blood smear negative) or to uninfected (Pf PCR negative) children. **P* < 0.05, as compared to children with asymptomatic (blood smear positive) infection. IP-10, IFN- γ -induced protein 10; sTNF-R1, soluble TNF receptor 1; sTNF-R2, soluble TNF receptor 2; sBAFF, soluble B cell activating factor, CRP, C-reactive protein.

FcγR-mediated phagocytosis pathway		Average Normalized Expression		Log₂ Fold Change	Adjusted P value
		AM	6wk		
FCGR1A	Fc fragment of IgG receptor Ia	158.6	38.2	1.98	0.0001
VAV1	vav 1 guanine nucleotide exchange factor	1124.2	651.5	0.76	0.0014
PTPRC	protein tyrosine phosphatase, receptor type, C	107.7	58.3	0.85	0.0019
RAC1	ras-related C3 botulinum toxin substrate 1	1360.5	807.2	0.70	0.0305
FCGR3A	Fc fragment of IgG receptor IIIa	13578.0	7010.5	0.82	0.0337
MAPK1	mitogen-activated protein kinase 1	1208.1	907.4	0.41	0.0467
CDC42	cell division cycle 42	6731.7	5242.3	0.36	0.0473
Staphylococcus aureus infection pathway		Average Normalized Expression		Log₂ Fold Change	Adjusted P value
		AM	6wk		
C3	complement component 3	136.8	60	1.08	0.0216
FCGR1A	Fc fragment of IgG receptor Ia	158.6	38.2	1.98	0.0001
C1QC	complement component 1, q subcomponent, C chain	732.6	36.2	3.85	8.2E-16
ITGB2	integrin, beta 2 (complement component 3 receptor 3 and 4 subunit)	13568.2	9815.6	0.45	0.0333
C2	complement component 2	209.2	131	0.79	0.0473
C3AR1	complement component 3a receptor 1	1880.3	1120.7	0.65	0.0491
C1QB	complement component 1, q subcomponent, B chain	2068.3	184	3.44	1.8E-15
C1QA	complement component 1, q subcomponent, A chain	1456.1	233.1	2.61	2.1E-09
FCGR3A	Fc fragment of IgG receptor IIIa	13578.0	7010.5	0.82	0.0337

Supplemental Table 4. Pathway enrichment during acute malaria compared to recovery.

Monocytes were negatively selected from PBMC collected during acute uncomplicated malaria (AM) and upon recovery (6wk), and targeted digital RNA sequencing was performed to analyze expression of 508 genes involved in inflammation and immunity. Of these 508 genes, 51 were overexpressed during AM. KEGG pathway enrichment was tested using the set of overexpressed genes compared to a background of all genes tested (significance threshold 0.01, minimum number of 3 genes). These 51 genes were significantly enriched for "Fc gamma R-mediated phagocytosis" ($P = 0.0007$; 7 genes) and "Staphylococcus aureus infection" ($P = 0.002$; 9 genes). Gene description source: HGNC Symbols.