

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

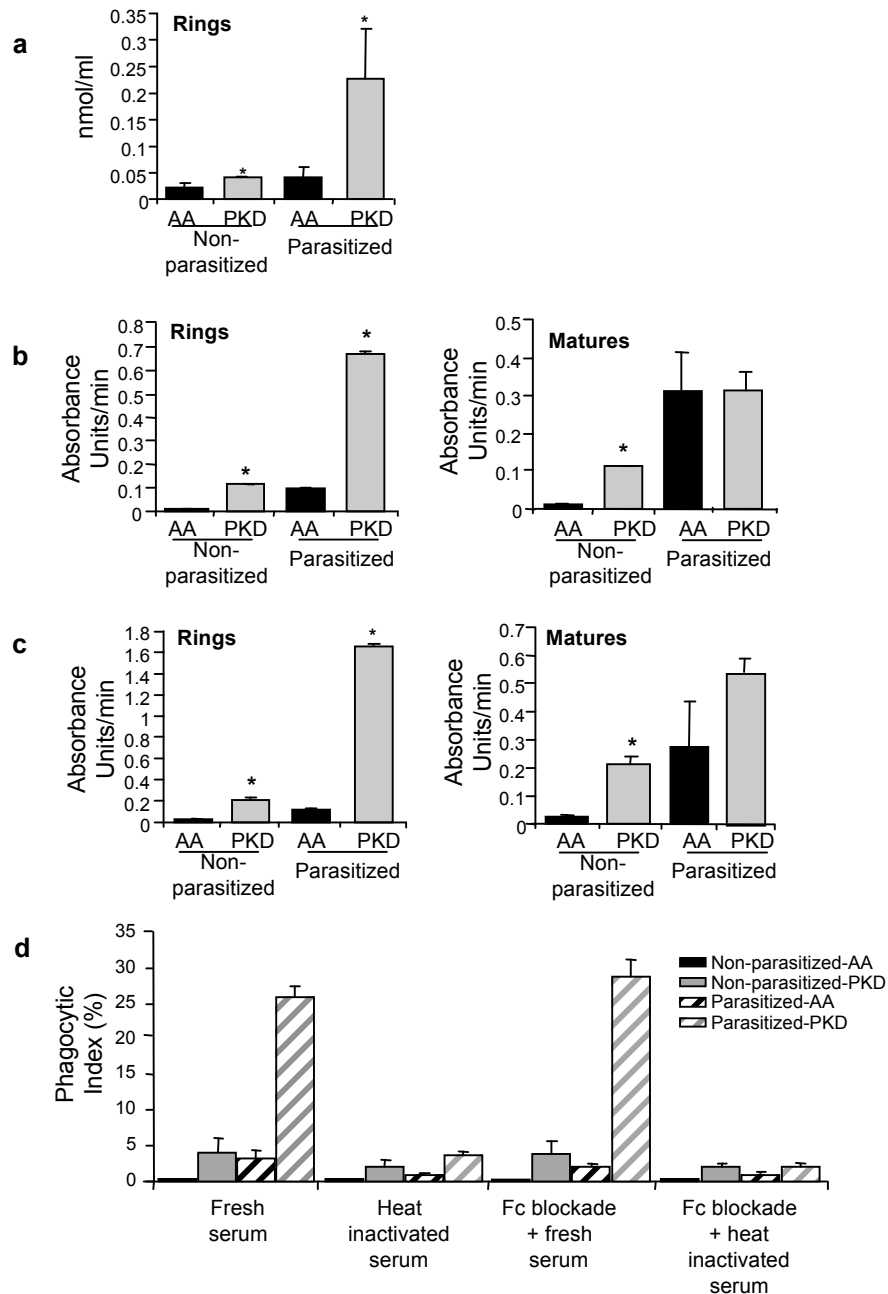
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Supplementary Appendix:

Supplementary Table 1. *P. falciparum* merozoites derived from homozygous PK-deficient erythrocytes display normal levels of invasion and maturation in control erythrocytes. Merozoites from *P. falciparum* parasitised PKD erythrocytes were allowed to invade and mature in control (AA) or PKD erythrocytes for two cycles of replication (See Supplementary Methods 1). Data are represented as mean (s.d.), n = 8. Statistical significance was assessed using a two tailed Student's t-test.

	First Cycle		Second Cycle	
	I (%)	M (%)	I (%)	M (%)
AA	2.4 (1.1)	1.5 (1.0)	9.9 (6.4)	7.6 (4.9)
PKD	1.3 (0.8)	0.8 (0.6)	2.6 (1.6)	2.4 (1.6)
P	0.0014		< 0.0001	

Abbreviation: I, invasion; M, maturation; AA, normal erythrocyte; PKD, pyruvate kinase deficient erythrocytes



Supplementary Figure 1. A higher level of complement binding on ring-stage parasitised PKD erythrocytes mediates their clearance by macrophages. (a) Membrane-bound hemichromes in hypotonic extracts of non-parasitised or ring-stage parasitised normal (AA) or PKD erythrocytes, expressed as nmol/ml of membrane. Binding of IgG (b) and complement C3c fragment (c) molecules, expressed as absorbance units/min/ 10^7 cells (See supplementary Methods 2). (d) Phagocytosis of ring-stage parasitised erythrocytes opsonised with fresh (complement containing) or heat-inactivated (no complement) human non-immune serum, with or without Fc receptor blockade using Fc fragments (See Methods). Data are shown as means \pm s.d. of 3 experiments. Statistically significant differences between normal (AA) and PKD erythrocytes were assessed by Student's *t*-test. *, $P < 0.0001$ for AA vs. PKD ($n = 5$ for a, b and c; and $n = 4$ for d).

Supplementary Methods 1. PK-deficient erythrocytes parasitised with *P. falciparum* clones ITG and 3D7 (mycoplasma-free) were synchronized at schizont stage (>95% purity)^{14,18}. To assess parasite invasion and maturation, purified schizonts at 0.5% parasitemia were co-cultured with normal (AA) or PKD erythrocytes at a hematocrit of 2% as previously described¹⁵. Slides were prepared from cultures at 24 h and 72 h to assess invasion and at 48 h and 96 h to assess maturation. Slides were stained with Diff-Quik, and 2000 erythrocytes were examined microscopically. Percent parasitemia was determined as follows: number of parasitised erythrocytes (matures or ring stage) divided by the total number of erythrocytes (non-parasitised and parasitised) x 100.

Supplementary Methods 2. *Membrane-bound hemichromes, autologous IgG and C3c fragment.* Bound hemichromes, IgG and C3c fragment were measured as described^{15,18}. For ring-stage parasitised erythrocytes the values were normalised to 100% parasitemia using the following formula: $I = (Tot - N \times n) / (1 - n)$ as described¹⁵, where I indicates the amount of bound IgG and C3c in 100% rings; Tot, amount of bound IgG and C3c in the whole culture; N, amount of bound IgG and C3c in non-parasitised erythrocytes; and n, fraction of non-parasitised erythrocytes. For mature-stage parasitised erythrocytes parasitemia was 5-10%.