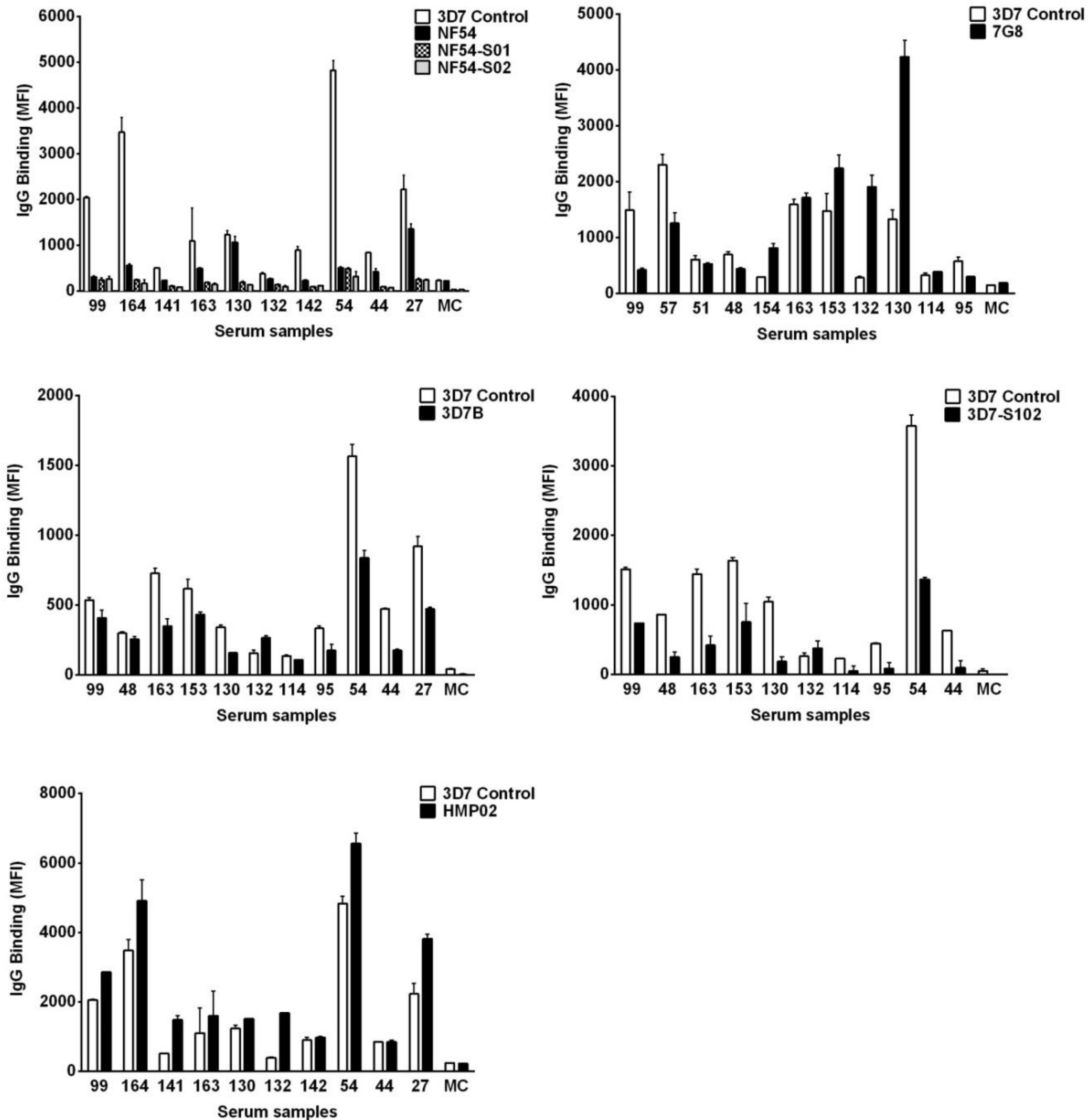

Figure S1 Antibody recognition among individual sera of *P. falciparum* parasitised erythrocytes from different parasite lines. The binding of antibodies from individual sera from Papua New Guinean adults to surface antigens expressed by *P. falciparum* parasitised erythrocytes from (A) *P. falciparum* NF54 (cultured cell bank), *P. falciparum* NF54-S01 and NF54-S02 (derived *ex vivo* from S01 and S02 at time of drug treatment), (B) *P. falciparum* 7G8 (cultured cell bank), (C) *P. falciparum* 3D7B (cultured cell bank), (D) *P. falciparum* 3D7-S102 (derived *ex vivo* from S102 at time of drug treatment) and (E) *P. falciparum*

HMP02 (*ex vivo* cell bank). IgG binding levels are expressed as geometric MFI for all graphs and bars represent median and interquartile ranges of samples tested in duplicate. There was minimal reactivity observed among sera from non-exposed Melbourne controls. Note the different y-axis scales.

Table S1. Symptoms reported in study participants.[a]

	Symptom	Malaria -mild	Malaria -moderate	Malaria -severe	Unrelated	Total
Study 1 n=2	Headache	0	0	0	3	3
	Arthralgia	0	0	0	1	1
	Flu-like Symptoms	0	0	0	3	3
	Eczema	0	0	0	1	1
	Transient asymptomatic arrhythmia	0	0	0	1	1
Study 2 n=2	Fatigue	0	0	0	2	2
	Shallow breathing	0	0	0	1	1
	Flu-like Symptoms	0	0	0	2	2
	Musculoskeletal injuries	0	0	0	1	1
Study 3 n=2	Fever	0	1	0	0	1
	Headache	0	1	0	0	1
	Abdominal discomfort	0	1	0	0	1
	Chills	0	1	0	0	1
	Microscopic	0	0	0	1	1

	haematuria					
Study 4	Headache	3	1	0	0	4
n=2	Myalgia	2	0	0	1	3
	Sweating	1	0	0	0	1
	Photophobia	1	0	0	0	1
	Nausea	1	0	0	0	1
	Funny taste	1	0	0	0	1
Study 5	Fever	0	3	0	0	3
n=2	Headache	2	1	0	0	3
	Musculoskeletal injuries	0	0	0	2	2
	Disorientation	1	0	0	0	1
	Flu-like Symptoms	1	0	0	0	1
	Cyst in throat	0	0	0	0	1

[a] Study 1: *P. falciparum* NF54 (cultured cell bank), Study 2: *P. falciparum* NF54 (cultured cell bank), Study 3: *P. falciparum* 7G8 (cultured cell bank), Study 4: *P. falciparum* 3D7B (cultured cell bank) and Study 5: *P. falciparum* HMP02 (*ex vivo* cell bank).

Table S2. Abnormal laboratory values in study participants.[a]

	Study 1 (n=2)	Study 2 (n=2)	Study 3 (n=2)	Study 4 (n=2)	Study 5 (n=2)
Thrombocytopenia	0	1[b]	0	0	0

[a] Study 1: *P. falciparum* NF54 (cultured cell bank), Study 2: *P. falciparum* NF54 (cultured cell bank), Study 3: *P. falciparum* 7G8 (cultured cell bank), Study 4: *P. falciparum* 3D7B (cultured cell bank) and Study 5: *P. falciparum* HMP02 (*ex vivo* cell bank).

[b] (109 x 10^9/L)

SUPPLEMENTARY METHODS

Measuring antibodies to the surface of P. falciparum parasitised erythrocytes by flow cytometry

Testing for IgG binding to the surface of parasitised erythrocytes (PE) by flow cytometry was performed as described previously (1). Samples collected from a cross-sectional study of Papua New Guinean adults (non-pregnant women and men) and non-exposed Melbourne donors were used (2). Ethics approval was obtained from the Medical Research Advisory Committee of PNG and the Human Research and Ethics Committee of Alfred Health. Written informed consent was provided by all participants. Briefly, PE containing fresh mature trophozoites at 0.2% haematocrit were consecutively incubated with test plasma or serum (1:10), polyclonal rabbit anti-human IgG (1:100, Dako), and Alexa-Fluor 488-conjugated donkey anti-rabbit IgG (1:500, Invitrogen) with ethidium bromide (1:1000, Bio-Rad), with washing between steps. All dilutions were performed in PBS with 0.1% Casein (Sigma Aldrich), and all incubations were at room temperature for 30 minutes. All samples were tested in duplicate. Data was acquired by flow cytometry (FACSVerse, BD Biosciences) and analysed using FlowJo software. IgG binding levels for samples were expressed as geometric MFI for PE, after subtracting the MFI of uninfected erythrocytes. Samples were designated antibody positive if the MFI was more than 3SD above the mean of reactivity of seen with non-exposed control serum.

Scanning electron microscopy

Square glass coverslips (22mm) were prepared by smearing a 0.1% solution of polyethyleneimine (PEI) and dried. Cell samples were incubated on PEI-coated or poly-L-lysine-coated coverslips and immersed in 2-2.5% glutaraldehyde for 1-2 hours. Coverslips were washed thrice in PBS for 10 minutes or rinsed in PBS followed by water and then

dehydrated in increasing concentrations of ethanol. Coverslips were dried in a Critical Point Dryer (Balzers Pfeiffer, Balzers, Leichtenstein) and mounted onto 25mm aluminium stubs with double-sided carbon tabs and then gold coated in a Dynavac "Xenosput" magnetron sputter coater. Cells were imaged with the Philips XL30 field emission scanning electron microscope (Philips, Eindhoven, Netherlands) at a voltage of 2kV or with the Quanta 200 FEG (FEI, Eindhoven, NL) at 10kV.

KAHRP expression assay

KAHRP expression was normalized to the expression of housekeeping gene fructose-bisphosphate aldolase (PF14_0425). Nucleic acid extraction was performed from 250 µl of packed red blood cells and where possible, specimens were stored in RNAprotect cell reagent (Qiagen, Australia). RNA extraction was performed using the RNeasy Plus Mini Kit (Qiagen, Australia) according to manufacturer's instructions. Alternatively, 250 µl packed red blood cells was stored in AL buffer and extracted using the QIAamp DNA Mini Kit (Qiagen Australia). DNA digestion was conducted on column using RNase-Free DNase kit (Qiagen, Australia) to eliminate genomic DNA. A one-step RT-PCR reaction mix (Qiagen, Australia) was used to performed qRT-PCR on RNA extracts. The primers targeting the KAHRP transcript were designed using Primer Express 3.0.1 software (Applied Biosystems Pty Ltd, Australia). Oligonucleotides for the housekeeping gene marker were modified for TaqMan hydrolysis probe chemistry, originally described by Salanti *et al.* (3). Each 25 µl PCR reaction contained 5.625 µl Qiagen One-Step RT-PCR Buffer, 1.125 µl of 10mM dNTPs, 1.0 µl of the RT-PCR enzyme mix, 0.45 µM of each primer (KAHRP2-Forward AAGTGCTCTAATAACTGCAATAATGGAAA, KAHRP2-Reverse TGTTCATGTTGCTTTTGTGCTAAA, PF3D7_1444800-Forward ATCTGGAGGACAATCAGAAGAAGAG and PF3D7_1444800-Reverse GCCATGTGTTCAATACTGAAGCTT), 0.18 µM of probes (KAHRP2-probe FAM-

TCCGGTGACTCCTTCGATTTCAGAAATAAGAGA-BHQ1 and PF3D7_1444800-probe FAM- CACCCATGGGCTTTAACCTTCTCTTACGG-BHQ1) and 5 µl of template RNA. Amplification was performed in a Rotorgene 3000 or 6000 (Qiagen, Australia) under the following conditions: 30 min reverse transcription at 50°C, 15 min incubation at 95°C followed by 45 cycles of 95°C for 15 sec and 60°C for 1 min. KAHRP and PF14_0425 quantification was performed in duplicate and the average copy number recorded. To control for potential DNA contamination, an additional PCR reaction was run following heat inactivation of the reverse transcriptase enzyme. A high load 3D7 culture was used as a positive control; all runs included no template negative control specimens.

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