Supplemental References

27 Ellman, R., Maxwell, C., Finch, R. & Shayo, D. Malaria and anaemia at different altitudes in the Muheza district of Tanzania: childhood morbidity in relation to level of exposure to infection. *Annals of tropical medicine and parasitology* **92**, 741-753 (1998).

28 Kabyemela, E. R., Fried, M., Kurtis, J. D., Mutabingwa, T. K. & Duffy, P. E. Decreased susceptibility to Plasmodium falciparum infection in pregnant women with iron deficiency. *J. Infect. Dis.* **198**, 163-166, doi:10.1086/589512 (2008).

29 Kurtis, J. D., Lanar, D. E., Opollo, M. & Duffy, P. E. Interleukin-10 responses to liver-stage antigen 1 predict human resistance to Plasmodium falciparum. *Infect Immun* **67**, 3424-3429 (1999).

30 Beier, J. C. *et al.* Plasmodium falciparum incidence relative to entomologic inoculation rates at a site proposed for testing malaria vaccines in western Kenya. *Am J Trop Med Hyg* **50**, 529-536 (1994).
31 Friedman, J. F. *et al.* Malaria is related to decreased nutritional status among male adolescents and adults in the setting of intense perennial transmission. *J. Infect. Dis.* **188**, 449-457, doi:10.1086/376596 (2003).
32 Gourley, I. S., Kurtis, J. D., Kamoun, M., Amon, J. J. & Duffy, P. E. Profound bias in interferon-gamma and interleukin-6 allele frequencies in western Kenya, where severe malarial anemia is common in children. *J. Infect. Dis.* **186**, 1007-1012, doi:10.1086/342947 (2002).
33 Gunasekaran, K., Jambulingam, P., Sadanandane, C., Sahu, S. S. & Das, P. K. Reliability of light trap sampling for Anopheles fluviatilis, a vector of malaria. *Acta Trop* **58**, 1-11 (1994).
34 Trager, W. & Jensen, J. B. Human malaria parasites in continuous culture. *Science* **193**, 673-675 (1976).
35 Bejon, P. *et al.* Effect of the pre-erythrocytic candidate malaria vaccine RTS,S/AS01E on blood stage immunity in young children. *The Journal of infectious diseases* **204**, 9-18, doi:10.1093/infdis/jir222 (2011).
36 Malkin, E. M. *et al.* Phase 1 clinical trial of apical membrane antigen 1: an asexual blood-stage vaccine for Plasmodium falciparum malaria. *Infection and immunity* **73**, 3677-3685, doi:10.1128/IAI.73.6.3677-3685.2005 (2005).
37 Long, C. Growth Inhibition Assay Proceedure. *http://www.niaid.nih.gov/labsandresources/labs/aboutlabs/lmvr/malariaimmunologysection/Pages/long.aspx#niaid_inlineNav_Anchor* (2013).
38 Lambros, C. & Vanderberg, J. P. Synchronization of Plasmodium falciparum erythrocytic stages in culture. *The Journal of parasitology* **65**, 418-420 (1979).
39 Rudlaff, R. M., Kraemer, S., Streva, V. A. & Dvorin, J. D. An essential contractile ring protein controls cell division in Plasmodium falciparum. *Nat Commun* **10**, 2181, doi:10.1038/s41467-019-10214-z (2019).
40 Pulcini, S. *et al.* Mutations in the Plasmodium falciparum chloroquine resistance transporter, PfCRT, enlarge the parasite's food vacuole and alter drug sensitivities. *Sci Rep* **5**, 14552, doi:10.1038/srep14552 (2015).
41 Cham, G. K. *et al.* A semi-automated multiplex high-throughput assay for measuring IgG antibodies against Plasmodium falciparum erythrocyte membrane protein 1 (PfEMP1) domains in small volumes of plasma. *Malar J* **7**, 108, doi:10.1186/1475-2875-7-108 (2008).
42 Oleinikov, A. V. *et al.* High throughput functional assays of the variant antigen PfEMP1 reveal a single domain in the 3D7 Plasmodium falciparum genome that binds ICAM1 with high affinity and is targeted by naturally acquired neutralizing antibodies. *PLoS Pathog* **5**, e1000386. Epub 1002009 Apr 1000317. (2009).
43 Oleinikov, A. V. *et al.* Effects of sex, parity, and sequence variation on seroreactivity to candidate pregnancy malaria vaccine antigens. *The Journal of infectious diseases* **196**, 155-164, doi:10.1086/518513 (2007).
44 Oleinikov, A. V. *et al.* A Plasma Survey Using 38 PfEMP1 Domains Reveals Frequent Recognition of the *Plasmodium falciparum* Antigen VAR2CSA among Young Tanzanian Children. *PLoS ONE* **7**, e31011, doi:10.1371/journal.pone.0031011 (2012).
45 Pardi, N., Muramatsu, H., Weissman, D. & Kariko, K. In vitro transcription of long RNA containing modified nucleosides. *Methods in molecular biology* **969**, 29-42, doi:10.1007/978-1-62703-260-5_2 (2013).
46 Thess, A. *et al.* Sequence-engineered mRNA Without Chemical Nucleoside Modifications Enables an Effective Protein Therapy in Large Animals. *Mol Ther* **23**, 1456-1464, doi:10.1038/mt.2015.103 (2015).
47 Weissman, D., Pardi, N., Muramatsu, H. & Kariko, K. HPLC purification of in vitro transcribed long RNA. *Methods in molecular biology* **969**, 43-54, doi:10.1007/978-1-62703-260-5_3 (2013).
48 Maier, M. A. *et al.* Biodegradable lipids enabling rapidly eliminated lipid nanoparticles for systemic delivery of RNAi therapeutics. *Molecular therapy : the journal of the American Society of Gene Therapy* **21**, 1570-1578, doi:10.1038/mt.2013.124 (2013).
49 Jayaraman, M. *et al.* Maximizing the potency of siRNA lipid nanoparticles for hepatic gene silencing in vivo. *Angewandte Chemie* **51**, 8529-8533, doi:10.1002/anie.201203263 (2012).

Supplementary Table 1 | Epidemiologic characteristics of resistant and susceptible individuals used in differential screening assays

Variable	Resistant	Susceptible	P value*
Number of subjects	12	14	-
Hemoglobin Phenotype (% Sickle trait)	25	14.3	0.918
Sex (% female)	25	50	0.190
Weeks of follow-up (median [IQR])	143 [23.5]	158 [16]	0.047
# Of blood smears from age 2-3.5 years (median [IQR])	14 [7.4]	21 [8.5]	0.0102
# Of positive blood smears from age 2-3.5 years (median [IQR])	2.1 [1]	5.8 [3.5]	0.0024
# Of anti-malarial treatment before 2 (median [IQR])	1.3 [1.1]	2.3 [3.1]	0.133
Pregnancy malaria (%)	25	21.4	0.300
Maternal age (yrs, median [IQR])	27 [8]	26.5 [6]	0.833
Birth Season (% in high season‡)	41.7	42.9	0.952
Subjects using bed net (%)	0	100	<0.001
# Of previous pregnancies (median [IQR†])	2 [2]	2.3 [2.1]	0.760
Parasite density (parasites per 200 WBCs§) at 2 year blood draw	0	0	1
Mean Parasite density (parasites per 200 WBCs) from age 0-2 years (median [IQR])	623 [952]	1400 [736]	0.1569
Mean Parasite density (parasites per 200 WBCs) from age 2 -3.5 years (median [IQR])	7.1 [2.9]	2258 [1569]	<0.001

* Comparisons of categorical variables by 2 tailed Fisher's exact test. Comparisons of continuous variables by two-sided Mann-Whitney U test

† Interquartile range

‡ May-October

§ White blood cells

Supplementary Table 2 | Parasite genes identified by differential bio-panning of phage display library. Results represent sequencing data from 100 phage clones.

Gene name	Gene ID & length in bp (Introns spliced out)	% Of Clones	Fragment size & nt. position in the gene
Glutamic acid-rich protein (GARP)	PF3D7_0113000 (2022)	37	465 (1558-2022)
Glutamic acid-rich protein (GARP)	PF3D7_0113000 (2022)	5	246 (1777-2022)
Glutamic acid-rich protein (GARP)	PF3D7_0113000 (2022)	2	801 (1222-2022)
Heat shock protein 110 (HSP110)	PF3D7_0708800 (2622)	2	650 (1764-2414)
Knob-associated histidine-rich protein (KAHRP)	PF3D7_0202000 (2312)	4	241(1309-1549)
Conserved Plasmodium protein	PF3D7_0418300 (2979)	2	643 (1811-2453)
Rhoptry-associated membrane antigen (RAMA)	PF3D7_0707300 (2115)	3	451 (953-1403)
High mobility group protein B1 (HMGPB1)	PF3D7_1202900 (294)	2	140 (153-294)
Plasmodium exported protein (PHISTc)	PF3D7_0936800 (1152)	3	303 (695-998)
Serine/threonine protein kinase, FIKK family	PF3D7_1039000 (3107)	1	390 (1156-1546)
Ornithine aminotransferase	PF3D7_0608800 (1245)	1	332 (914-1245)
18S ribosomal RNA	PF3D7_0531600 (2092)	26	670 (399-1068)
60S ribosomal protein L5, putative	PF3D7_1424100 (549)	11	460 (1-459)

Supplementary Table 3 | Primers used in construction of 3D7-PfGARP KD and 3D7-PfGARP KO parasites.

Oligo Number	Sequence
oJDD1027	gtacgcggccgcCCAAGTCCATTGACAGACG
oJDD4507	CTTgTGgTTgTCcCTcCTaGGgACaACgTTgACtCGaCCaATtGTACCTATTAATTGCTCTTGTATGGATAAG
oJDD2933	CTGCTGCTGAGTACTATCAAGTC
oJDD4279	CTACTTTGTTCTGATCATATG
oJDD4280	TTCTTTTTTGGGGGGCTTTCATG
oJDD4506	CaATtGGtCGaGTcAAcGTtGTcCCtAGgAGgGAcAAcCAcAAGAAGAAAATGGCAAAAATAGAAGAAGCAG
oJDD3566	tgcatgccatggTATTTTTGCATTTTTTCTTAAATTTC
oJDD3563	CAAAAAAGAAAAAATCTTAgatatcCCAAGTCCATTGACAGACGTAACTACACCAG
oJDD4507	CTTgTGgTTgTCcCTcCTaGGgACaACgTTgACtCGaCCaATtGTACCTATTAATTGCTCTTGTATGGATAAG
oJDD3561	catgcagcggccgcTGTCCTGTTTTATTTGTAATGTTTTATTTG
oJDD3562	TCTGTCAATGGACTTGGgatatcTAAGATTTTTTCTTTTTTGGGGGGCTTTC
oJDD3058	TTATATATAAGAACATATTTATTAAATCTAgaattcGGATTTCTACACATCTTGAGGTTT
oJDD4088	GCTCTAAAACtaggacgtgttaatgtagtacAATATTATATACTTAATATGAAATATG
oJDD3059	GTAAGGAGAAAATACCGCATCAGGCGCCAGCCTAGGTTTATGGTAGCCTTAAAAACTTCA
oJDD4089	TATAATATTgtactacattaacacgtcctaGTTTTAGAGCTAGAAATAGCAAGTTAA
oJDD4077	catgcgcggccgcATTCTATAAACCTGTTAGGAATAAAGGTTAG
oJDD4979	cttaagGTTTTCTGTATTTATATATATATATATATATATATTACGTTTTAAATATAC
oJDD5029	catggaattcCCAGAAGAGATAATCATAAG
oJDD4083	gatccctaggGGCTTGATTATTTTAAGGGAATAGAAC
caPfGARP	AAGAAGAAAATGGCAAAAATAGAAGAAGCAGAATTACAAAAACAAAAACATGTTGATAAAGAAGAAGATAAAAAAGAAGAAAGTAAAGAAGTTGAAGAAGAAA GTAAAGAAGTTCAAGAAGATGAAGAAGAAGTTGAAGAGGATGAAGAAGAGGAAGAGGAGGAAGAGGAAGAAGAAGAGGAGGAAGAAGAGGAAGAAGAGG AAGAGGAAGATGAAGTAGAAGAAGATGAAGATGATGCAGAAGAAGACGAAGATGATGCTGAGGAAGATGAAGATGATGCAGAAGAAGACGATGATGATGCTG AAGAAGATGATGATGATGCTGAAGAAGATGATGATGAAGATGAGGATGAAGATGAAGAAGAAGAAGATGAGGAAGAAGAAGAGGAGAGTGAAAAGAAA ATAAAAAGAAATTTAAGAAAAAATGCAAAAATA

Supplementary Text

Protein processing of PfGARP

P. falciparum exports proteins to the RBC cytoplasm and membrane using the *Plasmodium* export element (PEXEL) motif followed by its cleavage in the endoplasmic reticulum by plasmepsin V, as well as by other, non-PEXEL mediated mechanisms [1]. PfGARP encodes an amino terminal signal sequence/transmembrane region (aa 1-22) and an appropriately located PEXEL element (aa 48-52). To determine whether parasites process and cleave the PEXEL element, we probed parasite extracts using peptide-specific antisera generated against peptides that flank the PEXEL element (aa 31-48 and aa 504-522). As expected, both peptide-specific antibodies recognized full length (aa 1-673) recombinant PfGARP while only the antibodies raised against aa 504-522 recognized rPfGARP-A (aa 410-673). Only the antibodies raised against aa 504-522 recognized native PfGARP in trophozoite-infected RBCs confirming that the PEXEL element (and therefore the signal sequence/transmembrane domain) in PfGARP is processed and cleaved from the mature protein (**Extended Data Fig. 10**). Because mature PfGARP lacks a transmembrane domain and consensus sequences for glycophosphatidylinositol or palmitic acid anchors, the mechanism of attachment of PfGARP to the exofacial surface of the RBC membrane remains unknown but may include interactions with RBC or trophozoite-infected RBC surface proteins. Supporting this notion, recent evidence indicates that a highly charged 28 aa peptide from PfGARP (aa 417-444) containing 5 lysine-rich repeats is able to bind to Band 3 [2].

Identification and characterization of PfGARP

PfGARP was originally identified based on screening a *P. falciparum* cDNA expression library with uncharacterized sera obtained from adults living in Papua New Guinea [3]. In the same Tanzanian birth cohort used to clone and validate PfGARP in the present study, PfGARP was identified by RNAseq as one of only four parasite transcripts specifically upregulated in parasites infecting children compared to adults [4]. More recently, the short lysine-rich tandemly repeated sequences of PfGARP have been

identified as critical localization signals that traffic parasite proteins to the RBC membrane [5].

1 Boddey, J. A. *et al.* Export of malaria proteins requires co-translational processing of the PEXEL motif independent of phosphatidylinositol-3-phosphate binding. *Nature communications* **7**, 10470, doi:10.1038/ncomms10470 (2016).

2 Almukadi, H. *et al.* Human erythrocyte band 3 is a host receptor for Plasmodium falciparum glutamic acid-rich protein. *Blood* **133**, 470-480, doi:10.1182/blood-2018-07-865451 (2019).

3 Triglia, T. *et al.* Structure of a Plasmodium falciparum gene that encodes a glutamic acid-rich protein (GARP). *Molecular and biochemical parasitology* **31**, 199-201 (1988).

4 Vignali, M. *et al.* NSR-seq transcriptional profiling enables identification of a gene signature of Plasmodium falciparum parasites infecting children. *The Journal of clinical investigation* **121**, 1119-1129, doi:10.1172/JCI43457 (2011).

5 Davies, H. M., Thalassinos, K. & Osborne, A. R. Expansion of Lysine-rich Repeats in Plasmodium Proteins Generates Novel Localization Sequences That Target the Periphery of the Host Erythrocyte. *The Journal of biological chemistry* **291**, 26188-26207, doi:10.1074/jbc.M116.761213 (2016).