Human Cyclophilin B forms part of a multi-protein complex during erythrocyte invasion by *Plasmodium falciparum*

Prem Prakash¹,², Mohammad Zeeshan¹, Ekta Saini¹, Azhar Muneer¹, Sachin Khurana¹, Bishwanath Kumar Chourasia¹, Arunaditya Deshmukh¹, Inderjeet Kaur¹, Surabhi Dabral¹, Niharika Singh¹,³, Zille Anam³, Ayushi Chaurasiya³, Shikha Kaushik³, Pradeep Dahiya⁴, Md. Kalamuddin¹, Jitendra Kumar Thakur⁴, Asif Mohmmed¹, Anand Ranganathan²,³ & Pawan Malhotra¹

Invasion of human erythrocytes by *Plasmodium falciparum* merozoites involves multiple interactions between host receptors and their merozoite ligands. Here we report human Cyclophilin B as a receptor for PfRhopH3 during merozoite invasion. Localization and binding studies show that Cyclophilin B is present on the erythrocytes and binds strongly to merozoites. We demonstrate that PfRhopH3 binds to the RBCs and their treatment with Cyclosporin A prevents merozoite invasion. We also show a multi-protein complex involving Cyclophilin B and Basigin, as well as PfRhopH3 and PfRh5 that aids the invasion. Furthermore, we report identification of a de novo peptide CDP3 that binds Cyclophilin B and blocks invasion by up to 80%. Collectively, our data provide evidence of compounded interactions between host receptors and merozoite surface proteins and paves the way for developing peptide and small-molecules that inhibit the protein–protein interactions, individually or in toto, leading to abrogation of the invasion process.
Malaria remains a lethal disease in large parts of the world despite an effective drug treatment, increasingly as a result of the emergence of drug-resistant parasites. Its symptoms and pathology are a direct result of invasion of host erythrocytes by the *Plasmodium* merozoite, a complex process that requires coordinated interactions between host erythrocyte and parasite surface proteins, because of which it is an attractive target for vaccine and drug development. Although more than 50 merozoite surface antigens are expressed on *Plasmodium* merozoite surface, till date barely 7–10 possible interactions between them and their erythrocyte receptors have been well documented.

Of these, merozoite surface antigens from two main families: erythrocyte binding proteins (EBPs) and reticulocyte binding like protein (RH) have mainly been studied for their role in erythrocyte invasion. The parasite ligand PRfH5, for example, binds to Basigin, an interaction found to be essential for invasion by all tested *Plasmodium falciparum* strains. Basigin has also been shown to be a druggable target for anti-malarial interventions as anti-Basigin antibodies effectively block erythrocyte invasion by different strains of *Plasmodium*.

Basigin has been referred to under a variety of names—CD147, OX-47 antigen and CE9 in rat; gp42 in mice; HT7, neurothelin and 5A11 antigen in chickens. CD147 or Basigin, acts as an extracellular matrix metalloproteinase inducer that regulates a number of biological processes, such as spermatogenesis, lymphocyte responsiveness, and movement of monocarboxylate transporters. These multiple activities of Basigin involve a number of interacting proteins. Among several Basigin interacting proteins, Cyclophilins are an interesting class of proteins in terms of structural, functional, and medical implications. Cyclophilins were discovered as host cell receptors for the potent immunosuppressive drug, Cyclosporin A. Cyclophilins belong to the immunophilin class of proteins and some members of this family have been associated with parasitic diseases. Human malaria parasite *P. falciparum* encodes 13 immunophilins or immunophilin-like proteins; however, their exact functions are still unknown.

In the present study, we use bacterial two-hybrid assay to identify human Cyclophilin B as a receptor for PRfH3 and show that CypB is present on the RBC surface and binds to the merozoites. Conversely, anti-RhofH3 antibodies inhibit the binding of Cyclophilin B to the merozoite surface. We demonstrate a multi-protein receptor ligand interaction involving human CypB and Basigin, and *Plasmodium* PRfH5 and

![Diagram](image-url)

**Fig. 1** Cyclophilin B is a novel erythrocyte receptor for *P. falciparum* merozoite binding. **a** Parameters highlighting length, molecular weight (MW), isoelectric point (pI), number of cysteines and grand average of hydropathicity (GRAVY) of the identified host-pathogen interacting protein partners Cyclophilin B (CypB) and PRfH3-C. **b** Bacterial two-hybrid assay between identified host-pathogen interacting partners. Streaks of the identified prey protein from the bacterial two-hybrid experiment between PRfH3-C and human lung cDNA library on X-gal indicator plate. All streaks are labeled to represent genes cloned in pBTn and pTRGn. CFP10-pTRGn/empty pBTn is the negative control; CFP10-pTRGn/ESAT6-pBTn is the positive control. **c** Liquid β-galactosidase assay to measure relative enzymatic activity of co-transformant pairs. Relative Miller’s units (M.U.) of RhopH3-CpBTq and Cyclophilin B-pTRG (human lung cDNA library) is shown. **d** Localization of CypB on the RBC surface. Human erythrocytes were incubated with primary anti-CypB monoclonal antibody (mouse) followed by secondary alexa-fluor 488 conjugated goat anti-mouse IgG antibody (1:200) and confocal microscopy. **e** Binding of CypB on the merozoite surface. Merozoites were incubated with 25 µM recombinant CypB for 2 h followed by incubation with primary anti-CypB monoclonal antibody (mouse). The Merozoites were stained with alexa-fluor 488 conjugated goat anti-mouse IgG antibody (1:200; green) against primary antibody followed by confocal microscopy. Scale bar = 5 µm.
PfRhopH3. Additionally, by screening a codon-shuffled library we identify a 98 (aa)-long de novo peptide that inhibits the interaction between CypB and PfRhopH3 by binding to CypB and blocks invasion of the RBC by the Merozoites. Together, these results indicate that a multi-protein complex is formed involving CypB and PfRhopH3 and small molecules or peptides against these interacting proteins can act as potential drug candidates.

Results

CypB is a receptor for PfRhopH3. To look for novel host RBC and Plasmodium merozoite interactions, we used a bacterial two-hybrid\textsuperscript{22, 23} approach and screened a human tissue cDNA library against the PfRhopH3-C-terminal region (aa 617–865, Supplementary Fig 1). Plasmodium Rhotry proteins, as it has been shown previously, act as important ligands for host receptors during the invasion process\textsuperscript{24}. Specifically, PfRhopH3 has been shown to form a complex with the merozoite protein MSP1 and subsequently interact with erythrocyte Band 3 proteins to facilitate invasion\textsuperscript{25, 26}. On the basis of blue–white selection, a putative colony positive for interaction was selected and the isolated DNA sequenced. Sequence analysis showed the host interacting partner of PfRhopH3-C as the full-length (aa 1–208) human Cyclophilin B (Fig. 1a, Supplementary Table 4). The plasmids harbored in the selected colony were segregated, confirmed by PCR, and used to co-transform competent R1 Escherichia coli cells to verify the interaction. Two-hybrid plasmids expressing the Mycobacterium tuberculosis proteins ESAT6 and CFP10, and whose interaction has been well-documented previously\textsuperscript{27–29}, acted as the positive control. These interactions are shown in Fig. 1b, c. As Cyclophilin B possesses endoplasmic reticulum (ER) directed signal sequence and is secreted out in chondrocytes cell\textsuperscript{27, 28}, we analyzed its erythrocyte surface expression. As shown in Fig. 1d, immunolocalization analysis using anti-Cyclophilin B antibody stained the human RBC well, thereby demonstrating the expression of Cyclophilin B on the RBC surface. To investigate whether Cyclophilin B acts as a receptor for the binding of the merozoite to the RBC, we assessed the binding of Cyclophilin B protein to free P. falciparum merozoites. We have previously performed similar studies to show ICAM-4 binding to merozoites\textsuperscript{22}. Cyclophilin B showed significant binding on the merozoite surface (Fig. 1e), and co-localized with PfRhopH3 (Supplementary Fig. 2e), thus suggesting it to be a receptor for merozoite binding of human RBCs. Further, we developed an intensity measurement-based Cyclophilin B-merozoite binding assay. Briefly, P. falciparum free merozoites were incubated with 25 μM of Cyclophilin B protein. Intensity of Cyclophilin B binding on P. falciparum merozoite surface was quantified on Nikon fluorescent confocal microscopy using NIS element. Figure 2a shows Cyclophilin B binding intensity on four merozoites. For intensity measurements, the mean binding intensity for ten merozoites was calculated (Fig. 2a, b, Supplementary Table 1 and Supplementary Fig. 4).

PfRhopH3 acts as a ligand of CypB for merozoite binding. To confirm PfRhopH3 as a ligand involved in binding to Cyclophilin B on the RBC surface, we expressed and purified the C-terminal of PfRhopH3 protein (aa 617–865) in a heterologous E. coli expression system under denaturing condition (Supplementary Fig. 2a). Recombinant PfRhopH3-C protein was subsequently refolded by dialyzing it against a decreasing concentration of the denaturant. Antibodies were raised against the refolded

Fig. 2 PfRhopH3 protein is a ligand for Cyclophilin B protein during merozoite binding. a Binding of Cyclophilin B on Merozoite surface. Binding shows binding of CypB on merozoite surface without treatment with any antibody. anti-Rap2b + CypB and anti-RhopH3-C + CypB show binding after treatment with anti-Rap2b and anti-RhopH3 antibody respectively. Intensity for each merozoite is mentioned at the top. Images with histograms represent only the intensity of the bound CypB protein. Anti-RhopH3-C antibody interferes with binding of CypB on merozoites, signifying that PfRhopH3 is a ligand for host Cyclophilin B. Scale bar = 5 μm. b Graph adapted from Supplementary Table 1 showing the average of binding intensity of CypB on 10 different merozoites, with error bars representing the standard deviation. All values were tested for significance using a two-tailed unpaired Student’s t-test. **<P 0.01.
PfRhopH3-C protein (Supplementary Methods). Supplementary Fig. 2b shows the differential migration of refolded PfRhopH3-C protein on SDS and Native PAGE under reducing and non-reducing conditions, suggesting proper folding of the protein. We next assayed the binding of recombinant PfRhopH3-C to the RBC surface. As shown in Supplementary Fig. 2d, PfRhopH3-C protein bound specifically to the RBCs and co-localized with human CypB on the RBC surface with a Pearson’s coefficient 0.62 (Fig. 3e). To determine whether PfRhopH3 is the ligand for Cyclophilin B, intensity measurement-based Cyclophilin B merozoite-binding assay was performed in the presence of anti-RhopH3 antibody. Briefly, free merozoites were incubated with anti-PfRhopH3 or anti-PfRAP2 antibodies where after washing, the treated merozoites were incubated with Cyclophilin B protein at a concentration of 25 μM. Cyclophilin B binding was assessed on the treated merozoites and compared with untreated merozoites. As shown in Fig. 2b, Supplementary Table 1, Supplementary Fig. 4, merozoites treated with anti-RAP2 antibody displayed similar binding intensity for Cyclophilin B as shown with the untreated control merozoites. However, more than 50% reduction in Cyclophilin B binding to merozoite surface was observed when the merozoite surface was treated with anti-RhopH3-C antibody, in comparison to merozoites treated with anti-RAP2 antibody or untreated cells (Fig. 2a, b, Supplementary Table 1, Supplementary Fig. 4). These results demonstrate that Cyclophilin B is the receptor for a merozoite surface localized Rhoptyr protein PfRhopH3, which is known to be part of a large PfRhopH complex involved in erythrocyte binding1, 29. We further performed in vitro interaction studies between Cyclophilin B and PfRhopH3 proteins. Both, ELISA-based binding analysis and Far-western analysis showed a tight and specific interaction between Cyclophilin B and PfRhopH3 proteins (Fig. 3a, b, Supplementary Table 1, Supplementary Fig. 4). Co-immunoprecipitation analysis further confirmed an interaction between Cyclophilin B and PfRhopH3-C (Supplementary Fig. 4). To quantify the interaction between Cyclophilin B and PfRhopH3, surface plasmon resonance (SPR) analysis was performed. Cyclophilin B displayed a strong interaction with PfRhopH3 with an equilibrium dissociation constant $K_D$ value of 1.6 x 10^{-7} M (Fig. 3d). Together, these results confirmed the involvement of a
novel receptor-ligand (Cyclophilin B-PfRhopH3) interaction in the invasion of human erythrocytes by *P. falciparum* merozoites.

CypB interacts with a critical merozoite receptor Basigin. Basigin is expressed in many cell-types such as hematopoietic cells including the human erythrocytes\(^1,\,3\). Basigin has been shown to be a signaling receptor for extracellular Cyclophilins A and B, through which Cyclophilins mediate chemotactic activities\(^1,\,3\). Cyclophilin A-Basigin interaction also regulates inflammatory responses in a number of diseases and is a target for new anti-inflammatory therapeutics\(^1,\,3\). To investigate whether Cyclophilin B and Basigin are co-expressed at the same time on human RBC surface, co-immunolocalization studies using anti-Cyclophilin B and anti-Basigin antibodies were performed. Both these antibodies stained human erythrocytes, thereby showing the existence and expression of both these receptors on RBC surface simultaneously (Fig. 4a). We next performed co-binding studies with these proteins on the merozoite surface. Cyclophilin B as well as Basigin bound to merozoites simultaneously (Fig. 4b, c), thereby indicating that these proteins bind to independent ligands on the merozoite surface that are, most likely, PfRhopH3 and PfRh5 respectively.

A multi-protein complex involves Basigin-CypB-PfRhopH3-PfRh5. As mentioned earlier, Basigin has been reported to bind with *Plasmodium* merozoite protein PfRh5\(^5,\,31\). A previous study has shown the co-localization of PfRh5 and PfRhopH3 on merozoite surface\(^32\) but their physical interaction has not yet been illustrated. Therefore, to determine whether Cyclophilin B specifically interacts with Basigin during *Plasmodium* merozoite invasion of RBCs and involved in complex formation, we carried-out protein-protein interaction analysis between Cyclophilin B and Basigin, between PfRh5 and PfRhopH3, and between Basigin and PfRh5. ELISA-based binding studies were performed as described earlier\(^22\). All of the above-mentioned interactions turned out to be positive and furthermore, robust, thereby indicating the existence of a multi-protein interaction between the two *P. falciparum* merozoite surface ligands and the two corresponding RBC surface proteins (Supplementary Fig. 5a–d). Further, co-localization of PfRhopH3 and PfRh5 was confirmed on the merozoite surface with a Pearson’s coefficient value of 0.62 (Supplementary Fig. 5g, h). Both these interactions were strong with \(K_D\) values of \(~4.3 \times 10^{-7}\) M and \(1.8 \times 10^{-8}\) M respectively. A previous study\(^8\) has reported a strong interaction between Basigin and PfRh5 with a \(K_D\) value of \(1.12 \times 10^{-6}\) M. We confirmed these interactions by Far-western blot and co-immunoprecipitation followed by Western blot analysis (Fig. 3b, c, Supplementary Fig. 5e, f). Together, these results strongly advocate an association between Cyclophilin B and Basigin on the RBC surface and between PfRh5 and PfRhopH3 on the merozoite surface. Based on these interaction analyses, we built a model shown in Supplementary Fig. 13.

A de novo peptide CDP3 binds CypB and blocks invasion. To investigate further the role of Cyclophilin B-PfRhopH3 interaction in the invasion of the RBC by *P. falciparum*, we next
screened a dicodon polypeptide synthetic library\textsuperscript{22, 33, 34} to identify potential de novo peptide/proteins binders to Cyclophilin B. A bacterial two-hybrid screening was performed between the Cyclophilin B gene cloned in pTRGnn plasmid, referred as CYPBpTRGnn, and a de novo dicodon polypeptide synthetic library cloned in pBTnn plasmid referred as DIEL-pBTnn (Supplementary Fig 7g). CFP10pTRGnn/empty pBTnn and CFP10pTRGnn/ESAT6pBTnn served as the negative and positive controls, respectively\textsuperscript{22} (Fig. 5a, b). A 98 aa long de novo protein named CDP3 (Supplementary Table 5, Supplementary Fig. 7a) was identified as an interactor of Cyclophilin B after several rounds of segregation, re-cloning and re-transformations. We have earlier used a similar approach to identify the role of host ICAMs in cell invasion by \textit{M. tuberculosis} and \textit{P. falciparum}\textsuperscript{22}. To further explore the interaction between CDP3 and Cyclophilin B, we cloned and expressed CDP3 in pMTSA plasmid. C-terminal Histidine-tagged CDP3 was purified from \textit{E. coli} BL21 (DE3) strain under denaturing conditions on Ni-NTA+ column and the...
protein was subsequently refolded by dialyzing it against a decreasing concentration of the denaturant. Purified and folded CDP3 protein was analyzed on SDS-PAGE and by western blot analysis (Supplementary Fig. 7b, c). Reciprocal ELISA-based interaction assays were performed between recombinant CDP3 and Cyclophilin B proteins. The binding of CDP3 with Cyclophilin B was found to be concentration dependent (Supplementary Fig. 7f). Further, we tested the efficiency of CDP3 to block the interaction between PfRhopH3 and Cyclophilin B by CDP3 was further studied using an ELISA-based interaction assay. Incubation of Cyclophilin B coated wells with different concentrations of recombinant CDP3 protein resulted in a dose dependent reduction in PfRhopH3 binding to Cyclophilin B (Supplementary Fig. 7g). Further, we tested the efficiency of CDP3 to block the interaction between CypB and Basigin in an ELISA-based binding assay. CDP3 was unable to block the interaction between PfRhopH3 and Cyclophilin B by CDP3 was further studied using an ELISA-based interaction assay. Incubation of Cyclophilin B coated wells with different concentrations of recombinant CDP3 protein resulted in a dose dependent reduction in PfRhopH3 binding to Cyclophilin B (Supplementary Fig. 7f). Further, we tested the efficiency of CDP3 to block the interaction between CypB and Basigin in an ELISA-based binding assay. CDP3 was unable to block the interaction between PfRhopH3 and Cyclophilin B by CDP3 was further studied using an ELISA-based interaction assay. Incubation of Cyclophilin B coated wells with different concentrations of recombinant CDP3 protein resulted in a dose dependent reduction in PfRhopH3 binding to Cyclophilin B (Supplementary Fig. 7f). Further, we tested the efficiency of CDP3 to block the interaction between CypB and Basigin in an ELISA-based binding assay. CDP3 was unable to block the interaction between PfRhopH3 and Cyclophilin B by CDP3 was further studied using an ELISA-based interaction assay. Incubation of Cyclophilin B coated wells with different concentrations of recombinant CDP3 protein resulted in a dose dependent reduction in PfRhopH3 binding to Cyclophilin B (Supplementary Fig. 7f). Further, we tested the efficiency of CDP3 to block the interaction between CypB and Basigin in an ELISA-based binding assay. CDP3 was unable to block the interaction between PfRhopH3 and Cyclophilin B by CDP3 was further studied using an ELISA-based interaction assay. Incubation of Cyclophilin B coated wells with different concentrations of recombinant CDP3 protein resulted in a dose dependent reduction in PfRhopH3 binding to Cyclophilin B (Supplementary Fig. 7f). Further, we tested the efficiency of CDP3 to block the interaction between CypB and Basigin in an ELISA-based binding assay. CDP3 was unable to block the interaction between PfRhopH3 and Cyclophilin B by CDP3 was further studied using an ELISA-based interaction assay. Incubation of Cyclophilin B coated wells with different concentrations of recombinant CDP3 protein resulted in a dose dependent reduction in PfRhopH3 binding to Cyclophilin B (Supplementary Fig. 7f). Further, we tested the efficiency of CDP3 to block the interaction between CypB and Basigin in an ELISA-based binding assay. CDP3 was unable to block the interaction between PfRhopH3 and Cyclophilin B by CDP3 was further studied using an ELISA-based interaction assay. Incubation of Cyclophilin B coated wells with different concentrations of recombinant CDP3 protein resulted in a dose dependent reduction in PfRhopH3 binding to Cyclophilin B (Supplementary Fig. 7f). Further, we tested the efficiency of CDP3 to block the interaction between CypB and Basigin in an ELISA-based binding assay. CDP3 was unable to block the interaction between PfRhopH3 and Cyclophilin B by CDP3 was further studied using an ELISA-based interaction assay. Incubation of Cyclophilin B coated wells with different concentrations of recombinant CDP3 protein resulted in a dose dependent reduction in PfRhopH3 binding to Cyclophilin B (Supplementary Fig. 7f). Further, we tested the efficiency of CDP3 to block the interaction between CypB and Basigin in an ELISA-based binding assay. CDP3 was unable to block the interaction between PfRhopH3 and Cyclophilin B by CDP3 was further studied using an ELISA-based interaction assay. Incubation of Cyclophilin B coated wells with different concentrations of recombinant CDP3 protein resulted in a dose dependent reduction in PfRhopH3 binding to Cyclophilin B (Supplementary Fig. 7f). Further, we tested the efficiency of CDP3 to block the interaction between CypB and Basigin in an ELISA-based binding assay. CDP3 was unable to block the interaction between PfRhopH3 and Cyclophilin B by CDP3 was further studied using an ELISA-based interaction assay. Incubation of Cyclophilin B coated wells with different concentrations of recombinant CDP3 protein resulted in a dose dependent reduction in PfRhopH3 binding to Cyclophilin B (Supplementary Fig. 7f). Further, we tested the efficiency of CDP3 to block the interaction between CypB and Basigin in an ELISA-based binding assay. CDP3 was unable to block the interaction between PfRhopH3 and Cyclophilin B by CDP3 was further studied using an ELISA-based interaction assay. Incubation of Cyclophilin B coated wells with different concentrations of recombinant CDP3 protein resulted in a dose dependent reduction in PfRhopH3 binding to Cyclophilin B (Supplementary Fig. 7f). Further, we tested the efficiency of CDP3 to block the interaction between CypB and Basigin in an ELISA-based binding assay. CDP3 was unable to block the interaction between PfRhopH3 and Cyclophilin B by CDP3 was further studied using an ELISA-based interaction assay. Incubation of Cyclophilin B coated wells with different concentrations of recombinant CDP3 protein resulted in a dose dependent reduction in PfRhopH3 binding to Cyclophilin B (Supplementary Fig. 7f). Further, we tested the efficiency of CDP3 to block the interaction between CypB and Basigin in an ELISA-based binding assay. CDP3 was unable to block the interaction between PfRhopH3 and Cyclophilin B by CDP3 was further studied using an ELISA-based interaction assay. Incubation of Cyclophilin B coated wells with different concentrations of recombinant CDP3 protein resulted in a dose dependent reduction in PfRhopH3 binding to Cyclophilin B (Supplementary Fig. 7f). Further, we tested the efficiency of CDP3 to block the interaction between CypB and Basigin in an ELISA-based binding assay. CDP3 was unable to block the interaction between PfRhopH3 and Cyclophilin B by
CypB and CypA share a high sequence similarity\textsuperscript{15}, ELISA-based preliminary interaction studies were performed between CypA/ PIRhopH3-C, CypA/CDP3, and CypA/BSG proteins. The \textit{M. tuberculosis} ESA\textsubscript{T}6 protein was used as the negative control. Results showed that, because of high primary sequence similarity between CypA and CypB, our proteins of interest also bind to CypA (Supplementary Fig. 12).

**CypB is a crucial receptor for merozoite invasion of the RBC.** As CDP3 was able to potently block the interaction between Cyclophilin B (on the human erythrocyte surface) and PIRhopH3 (on the merozoite surface), we assessed its potential to block the merozoite invasion of host RBCs using an in vitro \textit{P. falciparum} culture. To functionally characterize the role of CDP3, different doses of CDP3 were added at 2% hematocrit to synchronized and purified \textit{P. falciparum} (3D7 or Dd2 strains) schizont stage parasites in complete RPMI medium. After 42–48 h of incubation at 37 °C, newly formed parasites were scored. Concentration-dependent reduction in parasitemia was observed; at 50 μM, it was found to be \textasciitilde60 % (Fig. 6b).

CsA is a cyclic hydrophobic undecapeptide that binds Cyclophilins and has been shown to possess significant anti-parasitic properties including anti-malarial activity both in vitro as well as in vivo\textsuperscript{36–38}. Although the precise anti-malarial mode of action of CsA is not yet known, CsA has been proposed to bind \textit{Plasmodium}-encoded Cyclophilins and Cyclophilin-like proteins\textsuperscript{39}. To confirm the role of Cyclophilins in the merozoite invasion of RBCs, and to know whether CsA acts by blocking the interaction between CypB and PIRhopH3-C, we tested its inhibitory potential in an ELISA-based inhibition assay. Importantly, CsA blocked the interaction between CypB and PIRhopH3-C (Supplementary Fig. 8a). To test whether CsA can also block the interaction between RhopH3-C and CypB in vivo, a liquid β-galactosidase assay was performed using CsA as an inhibitor. The results showed that CsA is a potent inhibitor of CypB/PIRhoph3 interaction in an in vivo system, too (Supplementary Fig. 8b).

Further, we repeated the invasion assay using human RBCs treated with the drug Cyclosporin A. To find out whether Cyclophilins expressed on the human erythrocyte are important for the \textit{P. falciparum} merozoite invasion of RBCs, human RBCs were treated with different concentrations of CsA for 1 h and treated RBCs were added at 2% hematocrit to synchronized and purified \textit{P. falciparum} cultures at schizont stage in complete RPMI medium. After 42–48 h of incubation at 37 °C, newly formed parasites were scored. A concentration-dependent reduction in the erythrocyte invasion was observed after treatment of human RBCs with CsA, with a maximum reduction of \textasciitilde80% observed in RBCs treated with 100 μM of CsA (Fig. 6c).

A similar dose-dependent decrease in parasitemia was witnessed with three other strains of \textit{P. falciparum}: Dd2 (sialic acid dependent strain), HB3 (sialic acid-independent strain) and 7G8 (chloroquine resistant strain) (Fig. 6d). Simultaneously, we also tested the ability of CsA to block merozoite invasion by adding it directly in the culture during the invasion where it can bind to the RBC Cyclophilin B as well as merozoite Cyclophilins. As shown in Fig. 6e, CsA when added directly into the culture, showed better inhibitory potential; maximum reduction of parasitemia (\textasciitilde80%) was observed with 50 μM of CsA treatment. This indicates that upon direct addition to the culture, CsA may have bound to both, the RBC Cyclophilins as well as the merozoite Cyclophilins, in keeping with the findings of a previous report that showed reduced invasion efficiency for CsA treated merozoites\textsuperscript{40}. These results were reproducible over three independent experiments, each carried out in duplicates.

Further, we performed an in vivo experiment to study and confirm the effect of CsA on parasitemia and survival of Balb/C mice. CsA was injected at 10 mg kg\textsuperscript{-1} body weight per day for four consecutive days and the dose initiated when mice were injected with 10\textsuperscript{5} RBCs infected with \textit{P. berghei}. As shown in Supplementary Fig. 11a, b, CsA was able to abolish the parasitemia even till 15 days of infection as compared to the control; survival was found to be 100% 30 days post-infection. To be sure, CsA has previously been shown to cause erythropsis\textsuperscript{41} in the course of treatment in mice. That said, a molecular analogue similar to CsA that can specifically target the interaction between PIRhopH3 and Cyclophilin B would, we believe, be efficacious in blocking parasite invasion. Together, these results suggest Cyclophilins as clear potential targets for blocking the merozoite invasion of RBCs and advocate the development of new anti-parasitic agents based on the CsA and CDP3 scaffold.

**Discussion**

In summary, we have applied a bacterial two-hybrid screening approach to identify Cyclophilin B as a novel receptor for a well-known \textit{P. falciparum} surface protein, PIRhopH3. We confirmed the interaction between host Cyclophilin B and merozoite PIRhopH3 protein by employing a range of protein–protein interaction tools, such as protein–protein interaction assays, SPR, far-western co-immunoprecipitation and co-localization. We developed a Cyclophilin B merozoite binding assay and showed a reduction in Cyclophilin B binding on the merozoite surface in the presence of anti-PIRhoph3 antibodies, thereby confirming a receptor-ligand interaction between these two proteins. To investigate whether host Cyclophilin B does indeed play a role in merozoite invasion of human RBCs, we screened Cyclophilin B against a synthetic polypeptide library and identified a 98 amino acid long protein CDP3 that binds to Cyclophilin B and inhibits its biologically significant interaction with PIRhopH3. Importantly, addition of CDP3 inhibited the invasion of RBC by \textit{P. falciparum} merozoites. Next, we tested the ability of the drug CsA to block the interaction between CypB and PIRhopH3 in vitro and in vivo; in both the assays CsA blocked these interactions. Further, the ability of CsA to block the host Cyclophilin B on the RBC surface was tested. We found that it efficiently blocked the merozoite invasion of treated RBCs, thereby confirming that Cyclophilin B is an important receptor for the merozoite invasion of RBCs. On the other hand, Basigin or CD147, an important integral plasma membrane glycoprotein that has been implicated in a variety of physiological and pathological activities and in particular for its role in inflammation and in merozoite invasion of human RBCs, performs these multiple functions by interacting with several human proteins or partners\textsuperscript{4}. Basigin also functions as a signaling receptor for extracellular Cyclophilins A and B to mediate their chemotactic activities\textsuperscript{42}. In neuron cells, interaction of CypA or CypB with Basigin has been shown to trigger a Ca\textsuperscript{2+} influx that results in the activation of ERK1/2 kinases\textsuperscript{43}. Encouragingly, molecules that disrupt the interaction between Cyclophilins and Basigin are being developed for the treatment of diseases such as cancer, HIV, hepatitis C virus, and coronaviruses\textsuperscript{44, 45}. Since Basigin has been shown to be an important receptor for
merozoite invasion of RBCs, we tested the co-expression and interaction of Basigin and Cyclophilin B. Both Basigin and Cyclophilin B were found to be expressed at the same time on human RBC surface and interact with each other. We further showed an interaction between their ligands PfRh5 and PRhophH3 on merozoite invasion of RBCs. It is conceivable the interaction of CypB with Basigin is triggering a Ca\(^{2+}\) flux, known to be essential for \textit{P. falciparum} merozoite invasion \cite{46}. The parasite has been known to use multiple redundant pathways to gain entry into the RBCs \cite{47}. We had earlier shown how dose-dependent inhibition of the RBC surface protein ICAM-4 results in blocking of the invasion process \cite{48}. Inhibition of Basigin through anti-Basigin antibodies, as stated earlier, also leads to blocking of the parasite invasion \cite{49}. In this context, the elucidation of a multi-protein interaction at the host-pathogen junction consisting of Cyclophilin B, Basigin, PRhophH3, and PRh5, the first two host proteins while the next two parasite proteins, offers an important handle for targeting parasite invasion at multiple levels. Finally, the finding that inhibitors of Cyclophilin B and Basigin are able to prevent the invasion of \textit{P. falciparum} strains could provide an alternative option for future development of therapeutics.

**Methods**

**Reagents.** The source of bacteria and \textit{P. falciparum} strains, as well as plasmids and antibodies is provided in Supplementary Table 2.

**Ethics statement.** Group of female BALB/c mice at 4 weeks of age and 2 rabbits, obtained from ICGEB animal house facility were used for this study by following the institutional ethical committee guidelines. All animal experiments were conducted in accordance with the guidelines approved by the Institutional Animal Ethics Committee of ICGEB, New Delhi, India.

**Human blood culture.** Human blood culture in this study. Donor blood was obtained from Rotary blood bank (RBB), New Delhi, India. RBB is an ISO 9001:2008 certified blood bank established in 2002. It follows stringent screening procedures, careful documentation and Good Laboratory Practices for collecting, processing and testing blood.

**Dicoson library construction.** The Dicoson Library \cite{43, 44} was prepared as described previously. Briefly, a 20 µl ligation mix was prepared containing 100 ng of each of the fourteen P-5’ DNA dicodons (DCs). To this mixture was added 7.5% polyethylene glycol (v/v) and the contents heated gently to 55°C. Once the temperature was brought down slowly to 4°C, the DC mixture was incubated at 4°C for a further 24 h. 100 picomol of P-5’ libraries was obtained from Rotary blood bank (RBB), New Delhi, India. RBB is an ISO 9001:2008 certified blood bank established in 2002. It follows stringent screening procedures, careful documentation and Good Laboratory Practices for collecting, processing and testing blood.

**Human lung cDNA library.** The human lung cDNA library, cloned into the pTRG vector, was acquired commercially from Stratagene, CA, USA (Catalog: 982201).

**Identification of human cyclophilin B as a binder of PRhophH3-C.** To identify host interacting partners of \textit{P. falciparum} RhophH3-C, a Bacterial two-hybrid experiment was performed according to the protocol provided by the manufacturer (Stratagene). Briefly, plasmid pBTHn containing the PRhophH3-C gene and pTRG containing the Human lung cDNA library were co-transformed in R1 reporter cells. The plasmids were pelleted by using X-Gal indicator plates containing kanamycin (50 µg mL\(^{-1}\)), chloramphenicol (30 µg mL\(^{-1}\)), tetracycline (12.5 µg mL\(^{-1}\)), X-Gal (80 µg mL\(^{-1}\)), Isopropl β-D-1-thiogalactopyranoside (25 µM), and phenylthioflavine β-D-thiogalactoside (200 µM). A positive control was set up in the form of \textit{E.coli} (pBTn and CF1P10TRGm co-transformation), a well-documented \textit{M. tuberculosis} protein-protein interaction. Bacteria co-transformed with CF1P10TRGm DNA and empty pBTHn plasmid represented the negative control. The indicator plates were incubated at 30°C for 36 h. The test plates were screened for positive interactions based on development of blue colonies. The plasmids harbored in the blue colonies were segregated, confirmed and cloned back into their respective plasmids. Each plasmid in R1 competent cells verified to the cells. Finally, the partner pBTHn and pTRGm plasmids were sequenced and a BLAST analysis performed to identify the interacting protein. This was found to be Human Cyclophilin B (CYPB; Peptidylprolyl Isomerase B or PPIB). Similarly, a Bacterial two-hybrid experiment was performed to identify inhibitors of chosen target proteins from different functional libraries. Cyclophilin B (CYPB) gene, cloned in plasmid pTRGq, was used as a bait. A de novo DIEL dicoson library was cloned in pBTq vector and the plasmids used to co-transform the R1 strain. Blue colonies obtained were analyzed in the same manner as described above and the interacting partner of CypB was identified.

**Liquid β-galactosidase assays.** To confirm and quantify the intensity of protein–protein interaction, a Liquid β-Galactosidase Assay was performed as described previously. Statistical significance was ascertained by performing a Student’s t-test. P-values <0.01 were considered significant.

**Bacterial three-hybrid screens.** The three-hybrid screens were performed as described previously \cite{43, 44}. Briefly, PRhophH3-pBTqg and CYPBpTRGq plasmids were used to co-transform the R1 reporter strain and the strain plated on X-gal indicator plates. Competent cells were prepared of the blue colony that contained both the plasmids. To set up a bacterial three-hybrid screen, the CDP3 gene (sequence provided in Supplementary Table 6) was cloned into the pMTSA plasmid and CDP3pMTSA plasmid used to transform the R1 competent cells that contained both RhophH3-CpBpTqg and CYPBpTRGq plasmids. The transformation mixture was plated on X-gal indicator plates, both in the presence and absence of L-arabinose (1% v/v). The competent cells carrying the two plasmids, RhophH3-CpBpTqg and CYPBpTRGq, transformed with CDP3pMTSA plasmid as described above were treated as the Test set-up (that yielded white colonies in presence of L-arabinose). The same “interacting” strain when transformed with empty pMTSA plasmid served as the negative control. Plates were incubated at 30°C. Reversion of colony color from blue to white upon induction of CDP3 expression by L-arabinose indicated disruption of RhophH3-C and CYPB interaction. The basis and validation of the bacterial three-hybrid system has been described previously.

**Arabinose gradient liquid β-galactosidase assay.** The three-hybrid liquid β-galactosidase assay, with increasing concentrations of L-arabinose was performed in triplicates as described previously \cite{43, 44}. Briefly, the selected triple cotransformant and the corresponding negative control were grown to mid-log phase in the presence of 40 µM IPTG. Optical density (A600) of each culture was measured at 600 nm. Induction was carried out using varying Arabinose concentrations ranging from 0 to 2 mM. Growth of induced culture at 37°C/200 r.p.m. was allowed for 3 h. Volume of 500 µl aliquot of each culture was pelleted down. Colorimetric liquid β-galactosidase assay of all samples using ONPG substrate was carried out as described previously. To measure statistical significance, a Student’s t-test was performed. P-values <0.01 were considered significant. Western blot analysis of R1 cell culture lysate was performed for the expression of CDP3-His using anti-His antibody (anti- His mAb, HRP conjugated, 1 mg mL\(^{-1}\), 1: 3000 dilution).

**Cloning and purification of PRhophH3-C.** The C-terminal portion of PRhophH3-C gene (PRHophH3-C, coding for 617–865 aa) was codon-optimized (sequence provided in Supplementary Table 6) for bacterial expression and synthesized commercially (Gene Script USA) in pUC57 vector, with the gene cloned at the unique NdeI restriction site. PRHophH3-C gene was excised and cloned in, NdeI-cut dephosphorylated pMTSA plasmid. RhophH3-pMTSA, upon induction, expresses PRhophH3-C with a C-terminal hexa-Histidine tag (PRHophH3-C-His6). To obtain his-tagged PRHophH3-C protein, E. coli BL21(DE3) cells harboring RhophH3-CpMTSA plasmid were grown till mid-log phase and induced with 0.5% L-arabinose for 24 h with constant shaking at 25°C. Cells were subsequently collected and the pellet washed with PBS (pH 7.4). Cell pellet was resuspended in lysis buffer (500 mM NaCl, 2.7 mM KCl, 4.3 mM Na2HPO4, 1.47 mM KH2PO4, 8 M urea, 2 mM PMSF) and sonicated till a clear lysate was obtained. The lysate was centrifuged at 17,000xg for 1 h and the obtained pellet solubilized in solubilization buffer (150 mM NaCl, 2.7 mM KCl, 4.3 mM Na2HPO4, 1.47 mM KH2PO4, 8 M urea, pH 8.0) and incubated at 25°C for 12 h with continuous shaking. Solubilized sample was centrifuged at 20,000 xg for 1 h at 25°C. The resulting supernatant was filtered through 0.45 µm nitrocellulose filter and allowed to bind overnight to 10 ml of Ni-NTA superflow resin (50% (v/v) suspension in 25% alcohol) at 25°C with shaking at 10 rpm. The column was washed with 10-bed volumes of binding buffer (150 mM NaCl, 2.7 mM KCl, 4.3 mM Na2HPO4, 1.47 mM KH2PO4, 8 M urea, 10 mM imidazole, pH 8.0) and buffer B (150 mM NaCl, 2.7 mM KCl, 4.3 mM Na2HPO4, 1.47 mM KH2PO4, 8 M urea, 500 mM imidazole, pH 8.0). The purification was performed using an Agilent 2100 Bioanalyzer equipped with 2100 Array kit. The sample was loaded on to the column, eluted with 300 µl buffer B, and the bound protein eluted with 150 mM NaCl, 2.7 mM KCl, 4.3 mM Na2HPO4, 1.47 mM KH2PO4, pH 8.0, by gradual removal of urea from 8–0 M. Purified protein was analyzed by running on SDS-PAGE and...
western blot using anti-PfRhopH3 antibody (Rabbit polyclonal (1:5000), prepared in-house; unpublished work) and CFP1023 were used as negative controls for the immunoblotting experiments. For measuring kinetics of interaction, increasing concentrations of recombinant PfRhopH3-C, BSG, and CD147 were injected at a flow rate of 30 µl/min over the immobilized PfRhopH3 as well as the reference flow cell, at a flow rate of 30 µl/min. Surface regeneration and kinetics analyses were performed using the methods described above. The PicpQ4 protein was used as negative control for the SPR experiments (Supplementary Fig. 6).

**Surface plasmon resonance (SPR)**: SPR assays were performed according to the protocol described previously with minor modifications. Briefly, biotinylated surface chemistry of the biosensor chip (GE Healthcare). Acellate buffer (used as the immobilization buffer) for the SPR experiment. For measuring kinetics of interaction, increasing concentrations of recombinant PfRhopH3-C, BSG, and CD147 were injected at a flow rate of 30 µl/min over the immobilized PfRhopH3 as well as the reference flow cell, at a flow rate of 30 µl/min. Surface regeneration and kinetics analyses were performed using the methods described above. The PicpQ4 protein was used as negative control for the SPR experiments (Supplementary Fig. 6).

**Plasmidium falciparum culture and merozoite preparation.** *P. falciparum* 3D7, Dd2, HB3, and 7G8 strains (Supplementary Table 2) were cultured in complete RPMI (RPMI 1640 (Invitrogen, USA), 50 mg L−1 hypoxanthine (Sigma, USA), 0.5 g L−1 Albumax I ( Gibco, USA) and 2 g L−1 sodium bicarbonate (Sigma, USA) using O2 human erythrocytes (4% haematocrit) under mixed gas (5% O2, 5% CO2, and 90% N2) conditions. Cultures were synchronized in early ring stage with 5% sorbitol for at least two successive cycles. Synchronized and healthy parasites at schizont stage were purified by Percoll sedimentation. *P. falciparum* merozoites were isolated from infected erythrocytes as described earlier. Briefly, 12–15% tightly synchronized schizont stage layer was layered on 65% Percoll and centrifuged at 2000 rpm for 20 min at 4°C. The inter-phase of infected erythrocytes was recovered and washed twice in incomplete RPMI. These purified schizont stage parasites were grown again in the presence of E64 for 6–8 h, allowing schizosporation to mature without rupturing the erythrocyte membrane. The membrane of erythrocytes was ruptured by passing through a 1.2 µm Acrodisc 32-mm syringe filter. These purifed merozoites were recovered by centrifugation at 4000g for 10 min at RT.

**Localization of CypB and basigin on the RBC surface.** Uninfected O+ human erythrocytes were washed twice in 1XPBS and fixed in PFG (4% paraformaldehyde+0.0075% glutaraldehyde) for 20 min at room temperature. Fixed erythrocytes were washed again in 1XPBS and incubated with blocking buffer (4% BSA) for 2 h at RT on a rotary shaker. These erythrocytes were then incubated with monoclonal anti-CypB (rabbit; 1:50) antibodies for 2 h on ice. After washing with blocking buffer, erythrocytes were incubated with Alexa-fluor 488
conjugated goat anti-mouse IgG antibody (1:200) for Cy3 and Alexa-488 conjugated goat anti-rabbit IgG antibody (1:200) for Cy5. Binding of Cyclophilin B was assessed by incubating these merozoites with Alexa-488 conjugated goat anti-mouse IgG antibody (1:200) for Cy3. The fluorescent intensity of the Cy3 binding on merozoite surface was measured by using intensity profile setting on NIS element software.

Effect of cyclosporin A on invasion. To assess the effect of Cyclosporin A (CsA) on P. falciparum growth, synchronous cultures at late schizont stage with parasitaemia of 1% were treated with varying concentrations (1.25, 2.5, and 50 µM) of CsA in a 96-well culture plate in duplicate. Uninfected erythrocytes, infected erythrocytes alone, and infected erythrocytes treated with DMSO were taken as controls. Parasites were maintained for 40 h and stained with ethidium bromide (EtBr, 10µg/ml) for 5 min at RT following by blocking in blocking buffer (4% BSA) for 2 h at RT. These merozoites were then mounted with monocolonal anti-Cy3 (mouse; 1:50) and anti-BSG (rabbit; 1:50) antibodies for 2 h on ice. Merozoites were washed with blocking buffer and incubated with alexa-fluor 488 conjugated goat anti-mouse IgG antibody (1:200) for Cy3 and alexa-fluor 594 conjugated goat anti-rabbit IgG antibody (1:200) for BSA for 1 h at room temperature. DAPI was added during the last 10 min of this incubation period. The merozoites were washed again, smeared on slides and analyzed using a Nikon A1-R confocal microscope. In order to study the specificity and role of PRfRhopH3 in Cy3 binding, merozoites were pre-incubated with rabbit anti-PRfRhopH3 antibody and anti-Rap2b (1:25) separately for 15 min followed by the addition of 25 µM of Cyclophilin B. Binding of Cyclophilin B was assessed by incubating these merozoites with alexa-fluor 488 conjugated goat anti-mouse IgG antibody (1:200) for Cy3. The fluorescent intensity of the Cy3 binding on merozoite surface was measured by using intensity profile setting on NIS element software.

Effect of CD3 on invasion. To evaluate the potential of CD3 protein on parasite invasion inhibition in chloroquine sensitive, 3D7, and resistant Dd2 strains, synchronized parasites at schizont stage were seeded in 96-well culture plates maintaining 1% parasitemia and 2% hematocrit. Recombinant purified endotoxin free CD3 protein was added to the culture medium at varying concentrations of 1, 2.5, 5, 10, 15, 20, 25, 50 µM and the culture plate incubated at 37 °C. After 40 h, the parasites were stained with EtBr and the parasitemia calculated using FACS. Uninfected erythrocytes and infected erythrocytes alone were taken as controls. In another experiment, uninfected RBCs were treated with different concentrations (1, 2.5, 5, and 10 µM) of CD3 for 4 h at RT. After removal of unbound CD3, treated erythrocytes were used for fresh infection maintaining 2% hematocrit and 1% parasitemia. Uninfected erythrocytes and uninfected erythrocytes alone were taken as controls.

Erythrocyte binding assay of PRfRhopH3-C. Uninfected human erythrocytes were washed with incomplete RPMI media and incubated with 20 µg each of PRfRhopH3-C and MLH (Plasmodium nucleolar helicase) protein in incomplete RPMI media followed by fixation with PFG and subsequent blocking with BSA. Cells were incubated with anti-Rhoph3-C rabbit sera and then with anti-rabbit alexa-fluor 594 secondary antibody and DAPI. Cells were visualized using Nikon A1-R confocal microscope and the images were processed using NIS element software.

In vivo study of cyclophilin A in the murine model. Cryopreserved infected RBC blood from P. berghei infected mice were revived by injecting it intraperitoneally into Balb/C mice. Percentage of parasitemia was determined from Giemsa-stained blood smears prepared from the tail region. After achieving 10–12% parasitemia, 4-week-old Balb/C mice were treated with 107 IRBCs and mice were divided into two groups (five mice in each group). One group of mice was injected with buffer control and the second group of mice with CsA at 10 mg kg⁻¹ body weight of mice. Doses were initiated from the day zero of infection and followed up to day 4. The blood smears of treated mice were prepared from day 4 and mice were observed till day 30 for survival.

Data availability statement. The data supporting the findings of this study are available from the authors on request.
29. Kaneko, O. et al. Apical expression of three RhopH1/Clag proteins as components of the Plasmodium falciparum RhopH1 complex. Mol. Biochem. Parasitol. 143, 20–28 (2005).

30. Yurchenko, V. et al. Active site residues of cyclophilin A are crucial for its signaling activity via CD147. J. Biol. Chem. 277, 22959–22965 (2002).

31. Wright, K. E. et al. Structure of malaria invasion protein RHS with erythrocyte basigin and blocking antibodies. Nature 515, 427–430 (2014).

32. Rodriguez, M., Lustigman, S., Montero, E., Oksov, Y. & Lobo, C. A. PfRH5: a novel reticulocyte-binding family homolog of Plasmodium falciparum that binds to the erythrocyte, and an application of its receptor. PLOS ONE 3, e3300 (2008).

33. Chopra, S. & Ranganathan, A. Protein evolution by “codon shuffling”: a novel method for generating highly variant mutant libraries by assembly of hexamer DNA duplets. J. Mol. Biol. 10, 917–926 (2003).

34. Renshaw, P. S. et al. Structure and function of the complex formed by the human malarial parasite Plasmodium falciparum PfRh5: a novel reticulocyte-binding family homolog of Plasmodium falciparum that binds to the erythrocyte, and an application of its receptor. PLOS ONE 3, e3300 (2008).

35. Nickell, S. P., Scheibel, L. W. & Cole, G. A. Inhibition by cyclosporin A of rodent malaria in vivo and human malaria in vitro. Infect. Immun. 37, 1093–1100 (1982).

36. Page, A. P., Kumar, S. & Carlow, C. K. Parasite cyclophilins and antiparasite activity of cyclosporin A. Parasitol. Today 11, 385–388 (1995).

37. Hoffmann, K., Kakalis, L. T., Anderson, K. S., Armitage, I. M. & Handschumacher, R. E. Expression of human cyclophilin–40 and the effect of the His141→Trp mutation on catalysis and cyclosporin A binding. Eur. J. Biochem. 229, 188–193 (1995).

38. Marin-Menendez, A. & Bell, A. Overexpression, purification and assessment of rodospersory, M. Lustigman, S., Montero, E., Oksov, Y. & Lobo, C. A. PfRH5: a novel reticulocyte-binding family homolog of Plasmodium falciparum. Protein Expr. Purif. 78, 225–234 (2011).

39. Singh, S., More, K. R. & Chitnis, C. E. Role of calceinurein and actin dynamics in regulated secretion of microneme proteins in Plasmodium falciparum merozoites during erythrocyte invasion. Cell Microbiol. 16, 50–63 (2014).

40. Bobbala, D. et al. Effect of cyclosporine on parasitemia and survival of Plasmodium berghei infected mice. Biochem. Biophys. Res. Commun. 376, 494–498 (2008).

41. Yurchenko, V., Constant, S. & Bukrinsky, M. Dealing with the family: CD147 interactions with cyclophilins. Immunology 117, 301–309 (2006).

42. Bousol, S. et al. Evidence that intracellular cyclophilin A and cyclophilin A/CD147 receptor-mediated ERK1/2 signalling can protect neurons against in vitro oxidative and ischemic injury. Neurobiol. Dis. 25, 54–64 (2007).

43. Pushkarsky, T. et al. CD147 facilitates HIV-1 infection by interacting with basigin and blocking antibodies. EMBO J. 24, 2491–2498 (2005).

44. Wu, Y., Li, Q. & Chen, X. Z. Detecting protein–protein interactions by Far western blotting. Nat. Protoc. 2, 3278–3284 (2007).

45. Chugh, M. et al. Protein complex directs hemoglobin-to-hemozoin formation in Plasmodium falciparum. Proc. Natl Acad. Sci. USA 110, 5392–5397 (2013).

46. Boyle, M. J. et al. Isolation of viable Plasmodium falciparum merozoites to define erythrocyte invasion events and advance vaccine and drug development. Proc. Natl Acad. Sci. USA 107, 14378–14383 (2010).

52. Boyle, M. J., Wilson, D. W. & Beeson, J. G. New approaches to studying Plasmodium falciparum merozoite invasion and insights into invasion biology. Int. J. Parasitol. 43, 1–10 (2013).

Acknowledgements
We thank Dr Alan F. Cowman and Lin Chen, Infection and Immunity Division, The Walter and Eliza Hall Institute of Medical Research, Australia for providing PfRh5 protein and antibody and for helpful discussions and a critical reading of this manuscript. This work was supported by internal grants from the International Centre for Genetic Engineering and Biotechnology (ICGEB), New Delhi; Department of Biotechnology, Government of India grant BT/PR7427/BRB/10/1178/2013; and ICMR-CAR core grant, Special Centre for Molecular Medicine, JNU, New Delhi provided by the Indian Council for Medical Research, Government of India. P.P., M.Z., A.M. and S.E. were supported by the Department of Biotechnology, Government of India. The Malaria Biology group is supported by Programme Support Grant, Indo-Danish Research Grant, and Grand Challenge Vaccine Program (BT/PR5267/MEED/15/87/2012 & BT/IN/Denmark/13/SS/2014) by the Department of Biotechnology, Government of India. We thank Dr Sultan Tousif and Prof Gobardhan Das, SCMM, JNU, for help in conducting experiments related to this study. We thank Rotary blood bank, New Delhi, India, for providing human red blood cells for Plasmodium cultures. We also thank central instrumentation facility, NIPGR, New Delhi for SPR experiments. We also thank Manish Kumar, Advanced Instrumentation Research Facility (AIRF), Jawaharlal Nehru University, New Delhi for help in Circular Dichroism experiments.

Author contributions
P.P., A.M., A.R. and P.M. conceptualized and designed the experiments. P.P., N.S., S.K., A.C., Z.A. and S.K. performed two-hybrid and three-hybrid experiments, cloning experiments, and protein purifications. P.P. and S.K. carried out ELISA and Far western studies. P.P. and S.K. and I.K. performed co-immunoprecipitation experiments. P.D., P.P., S.K. and J.K.T. performed and analyzed the SPR data. P.P., M.Z., A.M. and E.S. performed and analyzed P. falciparum assays. M.Z., P.P., E.S. and S.D. carried out confocal studies. B.C. and A.D. generated antibodies. M.K and P.P. performed in vivo experiments. P.P., A.M., A.R. and P.M. Analyzed data and wrote the manuscript with help from all other authors.

Additional information
Supplementary Information accompanies this paper at doi:10.1038/s41467-017-01638-6.

Competing interests: The authors declare no competing financial interests.

Reprints and permissions information is available online at http://npg.nature.com/reprintsandpermissions/

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.