Immune evasion of *Plasmodium falciparum* by RIFIN via inhibitory receptors

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Malaria is among the most serious infectious diseases affecting humans, accounting for approximately half a million deaths each year¹. *Plasmodium falciparum* causes most life-threatening cases of malaria. Acquired immunity to malaria is inefficient, even after repeated exposure to *P. falciparum*, but the immune regulatory mechanisms used by *P. falciparum* remain largely unknown. Here we show that *P. falciparum* uses immune inhibitory receptors to achieve immune evasion. RIFIN proteins are products of a polymorphic multigene family comprising approximately 150–200 genes per parasite genome² that are expressed on the surface of infected erythrocytes. We found that a subset of RIFINs binds to either leucocyte immunoglobulin-like receptor B1 (LILRB1) or leucocyte-associated immunoglobulin-like receptor 1 (LAIR1). LILRB1-binding RIFINs inhibit activation of LILRB1-expressing B cells and natural killer (NK) cells. Furthermore, *P. falciparum*-infected erythrocytes isolated from patients with severe malaria were more likely to interact with LILRB1 than erythrocytes from patients with non-severe malaria, although an extended study with larger sample sizes is required to confirm this finding. Our results suggest that *P. falciparum* has acquired multiple RIFINs to evade the host immune system by targeting immune inhibitory receptors.

*P. falciparum* uses multiple immune evasion strategies throughout its developmental stages. Central to parasite survival is the invasion of erythrocytes, within which parasites replicate while being largely protected from immune attack. Blood-stage parasites cause malarial pathology and are the main targets of cellular and acquired antibody-mediated immunity⁴ ⁵. *P. falciparum*-infected erythrocytes (IEs) express members of the polymorphic PIEMP1, RIFIN and STEVOR protein families on their surfaces. PIEMP1 binds to endothelial receptors to sequester IEs from blood circulation, allowing parasites to escape splenic clearance⁶. The functions of RIFIN and STEVOR proteins are unclear, although single members of these protein families bind to uninfected erythrocytes to form so-called rosettes⁸ that may facilitate sequestration of infected erythrocytes and evasion of immune recognition in vivo⁹.

Host immune cells downregulate autoimmune responses by expressing diverse inhibitory receptors that are specific for self-antigens. Some viruses exploit these inhibitory receptors by mimicking corresponding ligands and thereby evade the immune response¹⁰. We hypothesized that *P. falciparum* similarly exploits inhibitory receptors by expressing parasite-derived ligands for these receptors on IEs to impair immune responses. We therefore analysed the binding of 13 human inhibitory receptors fused to Fc antibody fragments to erythrocytes infected with the *P. falciparum* laboratory strain 3D7 or with *P. falciparum* isolated from patients with malaria. In this screen, leucocyte immunoglobulin-like receptor B1 (LILRB1) was the only receptor that exhibited detectable increased binding to a small fraction of IEs (Extended Data Fig. 1).

Next, we investigated whether LILRB1 bound to erythrocytes infected with *P. falciparum* isolates acquired from seven Thai patients with malaria. We found that LILRB1 bound a large fraction (77%) of IEs from patient 6 and small fractions from patients 1 (32%) and 4 (15%) (Fig. 1a and Extended Data Fig. 2a). LILRB1, also known as ILT2, CD85j or LIR-1, is an inhibitory receptor of the LILR family, which are expressed on diverse immune cells and encoded by genes within the leucocyte receptor complex (LRC) region¹¹. LILRB1, which recognizes MHC class I molecules¹², binds to the MHC class I-like UL18² encoded by human cytomegalovirus, suggesting that the LILR family coevolved with microbial pathogens¹³ ¹⁴. Binding of LILRB1 to IEs was primarily observed during the middle trophozoite and schizont stages of *P. falciparum* development (Fig. 1b and Extended Data Fig. 2b). LILRB1 bound to subpopulations of IEs infected with four *P. falciparum* laboratory strains (Fig. 1c and Extended Data Fig. 2c). Erythrocytes do not express MHC class I molecules, indicating that a parasite-derived molecule served as an LILRB1 ligand.

The *P. falciparum* genome does not encode an MHC class I-like gene¹⁵. Thus, to identify putative LILRB1 ligands on IEs, we took advantage of the 3D7 strain of parasites, for which whole-genome sequence data are available. LILRB1–Fc bound to a small proportion of 3D7 IEs. We therefore enriched 3D7 IEs for LILRB1 binding using cell sorting and subsequently subcloned 3D7 parasites using limiting dilution to obtain LILRB1–binding (F2) and non-binding (D11) parasite clones (Fig. 1d). The proportions of LILRB1-binding IEs (F2) decreased during passage after cloning (Extended Data Fig. 2e), suggesting that the expression of putative LILRB1 ligands was regulated by transcriptional switching between members of a *P. falciparum* multigene family. To identify putative LILRB1 ligands on IEs, we conducted mass spectrometric analyses of proteins that co-precipitated with LILRB1–Fc from 3D7 F2 and D11 clones (Extended Data Fig. 3a). In two independent experiments, only one peptide (FHEYDER), which had a sequence that matched RIFIN sequences, was specific to F2 immunoprecipitates (Extended Data Fig. 3b, c and Supplementary Table 1).
To validate these findings, we generated transgenic parasites expressing RIFINs #3–5, which did not bind to LILRB1–Fc (LILRB1 RIFINs #3–5, PF3D7_0500400, PF3D7_1000500 and PF3D7_1254200) and uninfected erythrocytes were stained with LILRB1–Fc (red dot) and control–Fc (black dot), followed by Vybrant Green (VG). Percentages of LILRB1–binding IEs are shown. a. Analysis of IEs with a LILRB1–Fc fusion protein. Diagram of LILRB1–Fc binding to IEs. Schizont-stage P. falciparum IEs from patient 6 were stained with LILRB1–Fc (red dot) and control–Fc (black dot), followed by Vybrant Green. Percentages of LILRB1–binding IEs are shown. b. Different stages of P. falciparum IEs derived from patient 6 were stained with LILRB1–Fc, followed by Vybrant Green. Percentages of LILRB1–positive IEs are shown. c. Schizont-stage IEs from four P. falciparum laboratory strains were stained with LILRB1–Fc (red dot) and control–Fc (black dot), followed by Vybrant Green. Percentages of LILRB1–binding IEs are shown. d. Schizont-stage IEs from LILRB1–binding clone 3D7 (F2) and non-binding clone 3D7 (D11) were stained with LILRB1–Fc (red) and control–Fc (shaded grey). Data represent at least three independent experiments and the variabilities of data shown in a, b, and c are shown in Extended Data Fig. 2a, b and c, respectively.

RIFINs are encoded by 150–200 rif genes per parasite genome. The FHEYDER sequence is shared among 36 RIFINs in the 3D7 genome. Therefore, we cloned these rif genes to assess RIFIN binding to LILRB1. We cloned 25 and 6 rif genes with and without the FHEYDER sequence, respectively. Erythrocytes infected with 3D7 schizont-stage IEs from LILRB1-binding clone 3D7 (F2) and non-binding clone 3D7 (D11) were stained with LILRB1–Fc (red) and control–Fc (shaded grey). Data represent at least three independent experiments and the variabilities of data shown in a, b, and c are shown in Extended Data Fig. 2a, b and c, respectively.

The extracellular domain of RIFINs comprises a relatively conserved N-terminal region containing the FHEYDER sequence and a highly variable C-terminal region (Fig. 2b). LILRB1–Fc bound to the variable rather than the conserved region of LILRB1 RIFIN #1 expressed on 293T cells (Fig. 2c). Furthermore, recombinant LILRB1 RIFIN #1 protein, but not LILRB1 RIFIN #5, bound to LILRB1 expressed on transfected 293T cells (Fig. 2d and Extended Data Fig. 6). These results indicate that RIFINs interact directly with LILRB1 through specific amino acids or conformations within their variable region.

The LILR family comprises five activating, five inhibitory and one soluble form. LILRB1 shares more than 80% sequence identity with the activating LILRA2, which recognizes microbially cleaved immunoglobulins. LILRA2 and other LILRs did not bind to LILRB1.
RIFIN #1 expressed on 293T cells (Extended Data Fig. 7a). Similarly, LILRB1 was the only LILR family member that bound to erythrocytes infected with RIFIN #1-transgenic parasites (Extended Data Fig. 7b). No LILR bound to erythrocytes infected with RIFIN #5-transgenic parasites (Extended Data Fig. 7b). These data suggest that certain RIFINs evolved to interact specifically with LILRB1. The natural ligands for LILRB1 are HLA class I molecules. However, a search using Phyre2 indicated that neither LILRB1+ RIFINs nor LILRB1+ RIFINs share amino acid sequence similarities or predicted structural homology with HLA class I molecules. Furthermore, we could not identify amino acid sequence motifs characteristic of LILRB1+ RIFINs. Similar to the recent determination of the protein structure of PfEMP1–host receptor interactions, solving the structure of the LILRB1–RIFIN interaction may clarify molecular constraints that confer receptor-binding specificity and antigenic diversity on RIFINs.

LAIR1 is a gene within the LRC that encodes an inhibitory receptor for collagen, similar to LILRB1, suggesting that these genes are evolutionarily related. Unique antibodies isolated from individuals living in a malaria-endemic region contained a mutated LAIR1 insert that bound to RIFIN #6 (PF3D7_1400600) and RIFIN #7 (PF3D7_1040300), whereas binding of wild-type LAIR1 to these RIFINs was undetectable or weak. Three amino acid substitutions in the mutated LAIR1 (T67L/N69S/A77T) are responsible for its increased binding to RIFINs. Consistent with previous observations, mutated LAIR1 (T67L/N69S/A77T)–Fc bound to IEs from parasites transfected with RIFINs #6 and #7, but wild-type LAIR1–Fc did not (Extended Data Fig. 8a). However, when analysing the binding of wild-type LAIR1 to IEs from 15 RIFIN-transgenic parasite lines (Extended Data Fig. 4b), we found that LAIR1–Fc bound to IEs from parasites transfected with LILRB1–RIFIN #8 (PF3D7_1101100) (Fig. 2a). Moreover, wild-type LAIR1–Fc bound 5–78% of erythrocytes infected with parasites from Thai patients with malaria (Extended Data Fig. 8b).

When IE binding of both LILRB1–Fc and LAIR1–Fc was analysed using parasites isolated from three patients, IEs derived from patient 1 predominantly bound to both LILRB1–Fc and LAIR1–Fc, whereas IEs derived from patient 5 or patient 6 predominantly bound to LAIR1–Fc or LILRB1–Fc, respectively (Extended Data Fig. 8c). Similar to LILRB1+ RIFINs, structural homology to natural ligands for LAIR1 (collagens) was not predicted from our analysis of the LILRB1–binding (LAIR1+) RIFIN #8 sequence. Consistent with previous studies of RIFIN expression, these results suggest that RIFINs are expressed on the IE cell surface in a variegated manner and that P. falciparum evades immune attack through the interaction of RIFINs with various inhibitory receptors.

Next, we investigated whether RIFINs induced LILRB1- or LAIR1-mediated cell signalling using the nuclear factor of activated T cells (NFAT)–GFP reporter system, in which GFP expression is induced upon ligand interaction with LILRB1 or LAIR1. When LILRB1-reporter cells were co-cultured with immobilized recombinant RIFINs, the cells were activated and GFP expression was induced by LILRB1+ RIFIN #1 but not by LILRB1+ RIFIN #5 (Fig. 3b). Moreover, when LILRB1-reporter cells were co-cultured with erythrocytes infected with RIFIN-transgenic parasites, LILRB1-reporter cells were activated by IEs expressing LILRB1+ RIFIN #1 but not by IEs expressing LILRB1+ RIFIN #4 (Fig. 3c and Extended Data Fig. 2d). By contrast, IEs expressing LAIR1+ RIFIN #8 did not significantly stimulate LAIR1-reporter cells, whereas collagen, a host ligand for LAIR1, significantly stimulated the reporter cells (Extended Data Fig. 9a). Similarly, IEs derived from patient 1 and LAIR1+ IEs enriched from patient 4 IEs using cell sorting did not significantly stimulate LAIR1-reporter cells (Extended Data Fig. 9b, c). These results suggest that the LAIR1–RIFIN interaction was not sufficiently strong to deliver inhibitory signals that were detectable in this assay. However, the LAIR1–RIFIN interaction may transduce an inhibitory signal via LAIR1 in other in vivo situations.

LILRB1 is expressed on various types of immune cells, including T cells, B cells, NK cells and monocytes. LILRB1 expressed by primary human B cells is involved in suppressing B cell responses. We therefore examined the effects of RIFINs on B cells expressing LILRB1 (Fig. 3d). IEs expressing LILRB1+ RIFIN #1, but not LILRB1+ RIFIN #4, inhibited IgM-induced immunoglobulin M (IgM) production by primary human B cells in preparations of peripheral blood mononuclear cells (PBMCs) (Fig. 3e). Similarly, Chinese hamster ovary (CHO) cells expressing LILRB1+ RIFIN #1, but not LILRB1+ RIFIN #3, inhibited IgM production by PBMCs (Fig. 3f). We next investigated the effects of LILRB1+ RIFIN #1 and LILRB1+ RIFIN #5 on the cytolytic function of an LILRB1-expressing human NK cell line, NKL (Extended Data Fig. 9d). K562 cells, often used as targets for NK cells, stably transfected with LILRB1+ RIFIN #1 and LILRB1+ RIFIN #4 transgene. Percentages of GFP-expressing cells are shown. SSC, side-scatter light. d, Red and shaded-grey histograms indicate staining of primary human B cells from a healthy donor with an anti-LILRB1 antibody and control, respectively. e, Inhibition of human immunoglobulin M (IgM) production in PBMCs by IEs. Human PBMCs were co-cultured with IEs, and IgM was measured in culture supernatants (mean ± s.d.). Transgenic malarial parasites expressing LILRB1+ RIFIN #1, LILRB1+ RIFIN #4 or mock (GFP) are shown. Control indicates PBMCs alone. n = 3 technically independent samples. f, Inhibition of human IgM production by RIFIN-transfected CHO cells. PBMCs were co-cultured with CHO cells expressing LILRB1+ RIFIN #1, LILRB1+ RIFIN #4 and an unrelated gene (MDA3). Data represent the mean ± s.d. (n = 3 technically independent samples). *P < 0.05 (one-way ANOVA with Tukey’s post hoc test). Data represent at least three independent experiments, and the variability of data presented in c is shown in Extended Data Fig. 2d.
The interactions between immune receptors and these polymorphic rodent-specific species of Plasmodium falciparum is encoded by multigene families present in all human- and host immune system (Fig. 4b). RIFINs are unique to Plasmodium falciparum and as important targets of protective immunity. RIFINs play an active role in early infections and may serve as important targets of protective immunity. Antibodies against RIFIN subsets associated with severe malaria may be acquired early and before antibodies to malaria. A significantly higher number of RIFINs from patients with severe malaria (cerebral malaria or severe malarial anaemia) bound to recombinant LILRB1–Fc compared with IEs from patients without these severe forms of malaria (Fig. 4a). Variation in Plasmodium falciparum surface antigens in vivo is governed by the host's immune status. In areas with high Plasmodium falciparum transmission, immunity to severe malaria is acquired early in life and before the acquisition of clinical immunity. Antibodies against RIFIN subsets associated with severe malaria may be acquired early and before antibodies to other RIFINs. Therefore, we analysed IgG reactivity with recombinant proteins derived from the RIFIN-variable regions of LILRB1–RIFIN #1 and LILRB1–RIFIN #5 in plasma from 222 Tanzanian individuals. IgG acquisition was similar for the two RIFINs, the rapid acquisition of antibodies and marked increase in the proportion of individuals responding to the IEisenotypes was similar for the two RIFINs, the rapid acquisition of antibodies and marked increase in the proportion of individuals responding to the RIFINs and that LILRB1-binding RIFINs are adhesins implicated in severe Plasmodium falciparum malaria. The median number of IEs contributing to the pathogenesis of severe malaria. However, the sample size in this study was small (severe malaria, n = 9; non-severe malaria, n = 30), and further studies are required to clarify LILRB1's role in the pathogenesis of malaria. Immune escape strategies of malaria parasites. Efficient leukocyte Ig-like receptor signaling and crystal structure of disulfide-linked HLA-G dimer. J. Biol. Chem. 281, 10439–10447 (2006). Shiroishi, M. et al. Efficient leukocyte Ig-like receptor signaling and crystal structure of disulfide-linked HLA-G dimer. J. Biol. Chem. 281, 10439–10447 (2006).
1333-00220 (C.W.W.) and 4004-00624B (T.L.), The Lundbeck Foundation (T.L.)
and the United States National Institutes of Health (NIH R01HL130678, T.L.). F.S
was supported by the Taniguchi Memorial Fellowship program.

Author Contributions F.S. and K.H. performed most of the binding and
functional experiments, and analysed and discussed the data. T.Sa. performed
limiting dilution experiments and assisted with ligand identification. C.W.W.
performed binding and serological analyses in Tanzania. J.L. assisted with
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experiments and provided Thai P. falciparum isolates. E.T. prepared wheat germ
cell-free proteins. T.T. designed wheat germ cell-free protein expression system
and discussed the data. M.K. assisted with functional experiments. T.Su. assisted
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Tanzania and discussed the data. T.H. assisted with P. falciparum experimental
design and discussed the data. H.A. designed the study and analysed the data.
All authors contributed to the writing of the manuscript.

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www.nature.com/reprints. The authors declare competing financial interests;
details are available in the online version of the paper. Readers are welcome to
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Reviewer Information Nature thanks P. Preiser and the other anonymous
reviewer(s) for their contribution to the peer review of this work.
METHODS

The experiments were not randomized. The investigators were not blinded to allocation during experiments and outcome assessment.

Cells. Human embryonic kidney 293T and CHO cells were obtained from and authenticated by the RIKEN Cell Bank. NKL cells31 were a gift from L. L. Lanier (University of California, San Francisco). The K562 cell line was obtained from the Cell Resource Centre for Biomedical Research, Institute of Development, Ageing and Cancer, Tohoku University. PBMCs were separated from the blood of healthy donors using Ficoll density gradient centrifugation (GE Healthcare). Each cell line was tested regularly for mycoplasma contamination using PCR.

Collection of *P. falciparum* parasites from Thai patients with malaria. All clinical *P. falciparum* isolates were acquired from patients living in the Mae Sariang district, Mae Hong Son Province in Thailand according to the ethical guidelines described below. Clonal parasite lines were established from field-isolated parasites using limiting dilution. All parasite strains were cultivated with human erythrocytes (type O blood, haematocrit (Ht) 2%, the Japanese Red Cross Blood Centre) in complete medium, which consisted of RPMI-1640 medium containing 20% AlbuMAX 1 (Life Technologies), 25 mM HEPES, 0.225% sodium bicarbonate and 0.38 mM hypoxanthine supplemented with 10 μg·mL⁻¹ gentamicin. Strains were incubated in an atmosphere containing 90% N₂, 5% CO₂ and 5% O₂.

*P. falciparum* culture. The *P. falciparum* strains 3D7, Dd2, 3D7 clones were obtained using this procedure. The 3D7 parasite cultures were enriched with sorting cells that bound to LILRB1–Fc fusion protein pre-mixed with anti-IgG Fc–APC antibodies. These enriched IEs were then subjected to limited dilution to obtain a single clone. In brief, the enriched parasites were synchronized at the ring stage using 5% d-sorbitol, and trophozoite- and schizont-stage parasites were enriched using Percoll density gradient centrifugation (GE Healthcare). Parasite cultures were tested routinely for mycoplasma contamination using PCR.

Cloning of *P. falciparum* 3D7 parasite. The 3D7 parasite cultures enriched by sorting IEs were used for the cloning of 3D7. 3D7 parasites were cultured with human erythrocytes in RPMI-1640 containing 10% human serum. Transgenic parasites were maintained in RPMI-1640 containing 10% human serum and 25 μg/mL mycophenolic acid (Sigma-Aldrich). Parasites were synchronized at the ring stage using 5% d-sorbitol, and trophozoite- and schizont-stage parasites were enriched using Percoll density gradient centrifugation (GE Healthcare). Parasite cultures were tested routinely for mycoplasma contamination using PCR.

Preparation of infected red cell ghosts. Preparatory steps to enrich expression of Fc receptors on the cell surface of CHO cells, plasmids encoding fusion proteins comprising RIFINs and the transmembrane and cytoplasmic domains of mouse PILRα or mock (human melanoma differentiation-associated protein 5 (MDA5)) were used to co-transfect CD8 into CHO cells. Stable transfectants of K562-RIFIN #1 (amino acid residues 166–275) and RIFIN #3 (amino acid residues 166–331) linked to the transmembrane and cytoplasmic regions of PILRα, respectively, were obtained by transducing the pMXs retroviral expression vector and PLAT-E retroviral packaging cells transfected with an amphotropic envelope as previously described.

Flow cytometry. The binding of human receptor–Fc fusion proteins to IEs was examined by staining IEs with Fc fusion proteins premixed with an allophycocyanin (APC)-conjugated anti-human IgG Fc antibody (109-136-098, Jackson ImmunoResearch Laboratories). For the PILRα–Fc fusion protein, cells were mixed with PILRα–Fc followed by APC conjugated anti-human IgG Fc antibody to decrease non-specific binding. For double staining of IEs with LAIR1–Fc and LILRB1–Fc fusion proteins, LAIR1–Fc or LILRB1–Fc was mixed with virus-infected IEs or reporter cells gated using FSC and SSC parameters (Supplementary Gating Strategy for Flow Cytometry Analysis). Flow cytometric analyses were conducted using BD FACSCalibur, LSR II and FACSVerse flow cytometers (BD Biosciences). Data were analysed using FlowJo software (FlowJo).

Preparation of infected red cell ghosts. *P. falciparum*-infected red cells were incubated for 15 min at 4°C in 40 volumes of hypotonic solution (RPMI-1640 diluted fivefold in water). Ghost cells were collected by centrifugation at 15,000 r.p.m. for 15 min and washed three times with a hypotonic solution.

Biointylation of LILRB1–Fc protein and immunoprecipitation. Purified AviTag®-AviSin®–Fc fusion protein was biotinylated using Bio-Avidin protein ligase (Avidity) (Extended Data Fig. 3a). Red-cell ghosts obtained from erythrocytes infected with the LILRB1–Fc or LILRB1–D11 clones at the schizont stage were incubated with biotinylated LILRB1–Fc fusion protein and subsequently treated with 0.25 mM 3,3-dithiobis(sulfosuccinimidyl propionate) (DTSSP, Thermo Fisher Scientific).
to generate crosslinks. Red-cell ghosts treated without LILRB1–Fc were used as controls. The red-cell ghosts were then washed in phosphate-buffered saline, solubilized by boiling in sample buffer in the absence of 2-mercaptoethanol and immunoprecipitated using streptavidin sepharose (GE Healthcare). The immunoprecipitated products were eluted using 50 mM dithiothreitol (DTT) and analysed using a liquid chromatography–tandem mass spectrometer (LC–MS/MS; LTQ Orbitrap, Thermo Fisher Scientific) after tryptic digestion. All MS/MS spectra data were analysed using Mascot software (Matrix Science) with the 3D7 sequence database. 

Generation of transgenic parasites. Transgenic parasites were generated as previously described41. In brief, fresh erythrocytes were transfected with the RIFIN–PIC5N5 plasmid via electroporation and subsequently infected with 3D7 parasites. Four days later, the parasites were cultured in RPMI-1640 containing pyrimethamine. Transfection of the rIF transgenes was controlled by the promoter for the Plasmodium berghei elongation factor-α. These RIFIN–PIC5N5 plasmids can be maintained stably in an episomal form in the parasite during multiple rounds of cell division. 

Quantitative PCR. Trophozoite-stage P. falciparum-infected erythrocytes were cultivated and subjected to RNA extraction using TRizol (Thermo Fisher Scientific) according to the manufacturer’s instructions. Contaminating genomic DNA was removed from extracted RNA using the TURBO DNA-free kit (Thermo Fisher Scientific) according to the manufacturer’s instructions. The absence of detectable DNA in extracted RNA was confirmed using PCR with a primer set specific for the seryl-tRNA synthetase gene as previously described42. The cDNA was synthesized using SuperScript III (Thermo Fisher Scientific) and random hexamers according to the manufacturer’s instructions. Quantitative PCR was performed for the rif gene transcripts using Power SYBR Green Master Mix (Thermo Fisher Scientific) with primers (5′-AGGATAAGATTGAAGCTGAGCA-3′ and 5′-AGCTCTGATCAGATGTGGTGTC-3′) located within the 3′ UTR of the P. berghei heat shock protein, downstream of the rif transgenes. The seryl-tRNA synthetase gene was used as an internal control. Relative expression was calculated using the ΔCt method. In brief, the expression level of each transgene was normalized to that of the internal control using their threshold cycle (Ct) values, and then the relative expression levels of eight different transgenes were calculated, defining the average of the RIFIN #1 expression level as 1.

Recombinant proteins. For fusion proteins were produced in 293T cells using transient transfection and purified using protein A sepharose (GE Healthcare) as previously described43. Recombinant RIFINs were produced in E. coli BL21 (DE3) via IPTG induction, followed by TALON metal affinity chromatography (Takara) purification and subsequent protein refolding. In brief, inclusion bodies were obtained from IPTG-induced cells by disruption using a sonicator and solubilized with 6 M guanidinium hydrochloride and subjected to TALON metal affinity chromatography and subsequent protein refolding. In brief, inclusion bodies were obtained from IPTG-induced cells by disruption using a sonicator and solubilized with 6 M guanidinium hydrochloride and subjected to TALON metal affinity chromatography and subsequent protein refolding.

Cytotoxicity assay. The human erythroleukaemia cell line K562, which serves as a target cell for NK cell killing assays, was stably transfected with LILRB1 RIFIN #1 or LILRB1 RIFIN #5 fused with transmembrane and cytoplasmic regions of PILRα and labelled with 15 μM Calcein AM (Thermo Fisher Scientific) in complete medium (RPMI-1640 without phenol red, supplemented with heat-inactivated 10% FCS) for 30 min at 37 °C. After being washed twice with complete medium, cells were adjusted to 5 × 10³ per 100 μl. The NK cell effector cells were washed twice with complete medium and mixed with target parental or rif-transfected K562 cells in a round-bottomed 96-well plate with effector:target (E:T) ratio ranging from 50:1 to 12.5:1 in triplicate. The assay was also performed for spontaneous and maximum release (target cells in 2% Triton X-100 containing complete medium). Cells were centrifuged at 100 g for 5 min and incubated at 37 °C in an atmosphere containing 5% CO₂ atmosphere for 4 h. After incubation, cells were centrifuged at 1,500 rpm for 2 min, and the fluorescence of the supernatants were measured using a TriStar LB941 (Berthold Technologies). Percent specific lysis was calculated as follows: [spontaneous release – spontaneous release]/[maximum release – spontaneous release] × 100.

Binding assay. Parasites collected from Tanzanian paediatric patients with malaria were isolated and cultured as previously described44. Binding assays were performed using freshly isolated parasites within 24 h of culturing in Petri dishes coated with LILRB1–Fc or LILRA2–Fc (as a control) as previously described45. Unbound parasites were washed away, whereas bound parasites were fixed with glutaraldehyde, stained with Giemsa and counted. Number of IE s specifically bound to LILRB1–Fc (relative number of IE s) was calculated by subtracting the number of IE s binding to control-Fc from that of LILRB1–Fc. Cerebral malaria and severe anaemia were defined according to a Blantyre coma score <3 and blood haemoglobin <5 g dL⁻¹, respectively, as previously described42. No statistical method was used to specify sample size.

Serological analyses. For this study, 222 plasma samples from children and adults, aged 6 months to 60 years, were collected in Mkokola, Tanzania in 2004. Frozen samples were thawed and analysed for the presence of IgG reacting with recombinant RIFIN as previously described46. Antibody reactivity was measured in a bead-based LumineX assay, using beads coated with the variable regions of LILRB1 RIFIN #1 (PF3D7_1254800), LILRB1 RIFIN #5 (PF3D7_1254200) and GLURP R2, which was used as a marker of exposure. The cut-off was determined according to mean reactivity + 2 s.d. of 43 European donors never exposed to malaria.

Ethics approval. Ethics approval for the collection of parasite-infected blood from Thai patients was granted by the Research Ethics Committees of the Faculty of Medicine, Chiang Mai University (permission number: 187/2554) and the Department of Medicine, Mie University, Japan (permission number: 1312). The work was conducted in compliance with all relevant ethical standards and regulations governing research involving human samples. Written informed consent was obtained from all patients or the parents or guardians of paediatric patients. Erythrocytes were obtained from the Japanese Red Cross (research ID: 250143). Written informed consent was obtained from the parents or legal guardians of the children. All patients received treatment according to national guidelines. The study protocols were reviewed and approved by the National Health Research Ethics Committee (NatHREC), hosted by the National Institute for Medical Research (NIMR) of Tanzania (reference no. NIMR/HQ/R.8c/ Vol.II/436), and the methods used in this study were performed in accordance with the approved guidelines.

Data availability. Source data for Figs 3, 4 and Extended Data Figs 2, 5, 6, 9, 10 are provided with the paper. All data are available from the corresponding author upon reasonable request.

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33. Mitamura, T., Hanada, K., Ko-Mitamura, E. P., Nishijima, M. & Horii, T. Serum factors governing intraerythrocytic development and cell cycle progression of Plasmodium falciparum. Parasitol. Int. 49, 219–229 (2000).
34. Tanabe, K. et al. Allelic dimorphism-associated restriction of recombination in Plasmodium falciparum msp1. Gene 397, 153–160 (2007).
35. Satoh, T. et al. PILRα is a herpes simplex virus-1 entry coreceptor that associates with glycoprotein B. Cell 132, 935–944 (2008).
36. Shiratori, I. et al. Down-regulation of basophil function by human CD200 and human herpesvirus-8 CD200. J. Immunol. 175, 4441–4449 (2005).
37. Jiang, Y. et al. Transport of misfolded endoplasmic reticulum proteins to the cell surface by MHC class II molecules. Int. Immunol. 25, 235–246 (2013).
38. Jin, H. et al. Autoantibodies to IgG/HLA class II complexes are associated with rheumatoid arthritis susceptibility. Proc. Natl Acad. Sci. USA 111, 3787–3792 (2014).
39. Morita, S., Kojima, T. & Kitamura, T. Plat-E: an efficient and stable system for transient packaging of retroviruses. Gene Ther. 7, 1063–1066 (2000).
40. Iwanaga, S., Kato, T., Kaneko, I. & Yuda, M. Centromere plasmid: a new genetic tool for the study of Plasmodium falciparum. PLoS One 7, e33326 (2012).
41. Jiang, Y. et al. Evidence for in vitro and in vivo expression of the conserved VAR3 (type 3) Plasmodium falciparum erythrocyte membrane protein 1. Malar. J. 11, 129 (2012).
42. Furukawa, A. et al. Structural analysis for glycolipid recognition by the C-type lectins Mincle and MCL. Proc. Natl Acad. Sci. USA 110, 17438–17443 (2013).
43. Arumugam, T. U. et al. Application of wheat germ cell-free protein expression system for novel malaria vaccine candidate discovery. Expert Rev. Vaccines 13, 75–85 (2014).
44. Suenaga, T., Kohyama, M., Hirayasu, K. & Arase, H. Engineering large viral DNA genomes using the CRISPR–Cas9 system. Microbiol. Immunol. 58, 513–522 (2014).
45. Mkumbaye, S. I. et al. Cellulose filtration of blood from malaria patients for improving ex vivo growth of Plasmodium falciparum parasites. Malar. J. 16, 69 (2017).
46. Magistrado, P. A. et al. High efficacy of anti DBL4ε-VAR2CSA antibodies in inhibition of CSA-binding Plasmodium falciparum-infected erythrocytes from pregnant women. Vaccine 29, 437–443 (2011).
47. Turner, L. et al. Severe malaria is associated with parasite binding to endothelial protein C receptor. Nature 498, 502–505 (2013).
48. Turner, L. et al. IgG antibodies to endothelial protein C receptor-binding cysteine-rich interdomain region domains of Plasmodium falciparum erythrocyte membrane protein 1 are acquired early in life in individuals exposed to malaria. Infect. Immun. 83, 3096–3103 (2015).
Extended Data Figure 1 | Binding of Fc fusion proteins of inhibitory receptors to IEs. Erythrocytes infected with 3D7 (3D7 IEs), *P. falciparum* obtained from patient 1 (Patient 1 IEs), or uninfected erythrocytes were stained with Fc fusion proteins of inhibitory receptors. As a control, IEs were stained with APC-labelled anti-human IgG Fc Ab alone (Control). FSC, forward-scattered light. The experiments were replicated twice.
Extended Data Figure 2 | Variability and stability of LILRB1 binding to IEs and LILRB1 reporter activity. 

**a**, LILRB1 binding to schizont-stage *P. falciparum*-infected erythrocytes from patients with malaria in Fig. 1a. 

**b**, LILRB1 binding to *P. falciparum*-infected erythrocytes derived from patient 6 in Fig. 1b at the ring, mid-trophozoite and schizont stages. 

**c**, LILRB1 binding to schizont-stage *P. falciparum*-infected erythrocytes from the laboratory strains CDC1, K1, FCR3 and Dd2 shown in Fig. 1c. 

**d**, GFP expression in LILRB1-expressing reporter cells upon stimulation with *P. falciparum*-infected erythrocytes in Fig. 3c. Data represent the mean ± s.d. of three independent experiments. 

**e**, Proportions of LILRB1–Fc–binding erythrocytes infected with clone 3D7-F2 were analysed during 5 weeks of culture. The experiment was performed once.
Extended Data Figure 3 | Identification of the LILRB1 ligand.

a. Diagram of LILRB1 ligand identification. A putative LILRB1 ligand was immunoprecipitated from IE ghosts using an LILRB1–Fc fusion protein and was identified using mass spectrometry analysis. The observed m/z values of b-ions (red) and y-ions (blue) in the MS/MS spectra of the peptide FHEYDER present in reversed phase high performance liquid chromatography (RP-HPLC) fractions of trypsin digests of LILRB1 precipitates from IEs infected with F2 clones. The experiments were replicated twice. c. The observed m/z values of b-ions and y-ions in the MS/MS spectra of the peptide FHEYDER present in RP-HPLC fractions of trypsin digests of LILRB1 precipitates. The predicted m/z values are shown for comparison. The differences between the m/z values for observed ions and the predicted values are shown.
Extended Data Figure 4 | Screening of RIFINs that bound to the LILRB1–Fc or LAIR1–Fc fusion protein. a, IEs of 3D7 carrying RIFIN transgenes were stained with the LILRB1–Fc fusion protein. RIFIN transgenes are indicated. Red and shaded-grey histograms indicate staining with LILRB1–Fc and control–Fc fusion proteins, respectively. The presence of the FHEYDER sequence in each RIFIN is indicated in the figure. Representative data from independent analyses are shown. Therefore, the proportions of IEs bound to Fc fusion proteins and the levels of Fc fusion protein binding IEs may not be comparable among different RIFINs. All experiments were replicated twice.

b, IEs of 3D7 carrying RIFIN transgenes were stained with the LAIR1–Fc fusion protein. RIFIN transgenes are indicated. Red and shaded-grey histograms indicate staining with LAIR1–Fc and control–Fc fusion proteins, respectively. The presence of the FHEYDER sequence in each RIFIN is indicated in the figure. Representative data from independent analyses are shown. Therefore, the proportions of IEs bound to Fc fusion proteins and the levels of Fc fusion protein binding IEs may not be comparable among different RIFINs. All experiments were replicated twice.
Extended Data Figure 5 | Expression of RIFINs in transgenic malaria parasites. a, The rif transgene transcript levels normalized to the internal control gene. The average of RIFIN #1 transcript levels was defined as 1. Data represent the mean ± s.d. (n = 3 technically independent samples). RIFIN #6 expression was lowest among the transgenes. However, cell surface expression of RIFIN #6 was detected using a mutated LAIR1–Fc fusion protein (Extended Data Fig. 8a), indicating that all the transgenes were sufficiently expressed at the transcript level. b, Western blot analysis of the expression of transfected C-terminally His-tagged RIFINs transfected into malaria parasites using an anti-His tag monoclonal antibody. The His-tagged RIFINs were detected at approximately equal levels (Supplementary Data). The expected molecular masses are 31.7 (RIFIN #1), 32.9 (RIFIN #2) and 34.8 (RIFIN #5) kDa. The experiment was performed once. c, P. falciparum-infected erythrocytes expressing C-terminally His-tagged RIFIN transgenes (RIFIN #1, PF3D7_1254800; RIFIN #2, PF3D7_0223100; and RIFIN #5, PF3D7_1254200) were stained with LILRB1–Fc (red) and control–Fc (shaded grey). The experiments were replicated at least twice.
Extended Data Figure 6 | Recombinant RIFINs. a, Binding of recombinant RIFINs to LILRB1 produced using a wheat germ cell-free protein expression system. 293T cells expressing transfected LILRB1 or LILRA2 were stained with recombinant His-tagged RIFINs that were produced using a wheat germ cell-free protein expression system. LILRA2 is an activating counterpart of LILRB1 and was used as a control. Red and blue histograms indicate binding of LILRB1 + RIFIN #1 and LILRB1 − RIFIN #5, respectively. The shaded-grey histogram represents an unstained control. The experiments were replicated at least twice. b, Production of recombinant RIFINs in E. coli. N-terminally His-tagged variable regions of RIFINs were expressed in E. coli and purified using TALON metal-affinity chromatography. Recombinant RIFINs were analysed using SDS-PAGE and Oriole staining. LILRB1 + RIFIN #1 and LILRB1 − RIFIN #5 are shown on the right and left, respectively. The experiments were replicated at least twice. c, CD spectra of recombinant RIFINs. Refolded and purified recombinant RIFIN #1 and #5 were subjected to CD spectral analysis. The spectra are shown as the mean residue ellipticity after subtracting the solvent background. RIFINs #1 and #5 exhibited CD spectra typical of well-folded proteins with α-helix (208 nm + 222 nm) and β-sheet (215 nm) structures. Prediction of the secondary structures of each RIFIN using the BeStSel server (http://bestsel.elte.hu/index.php) yielded α/β values of approximately 30%/10% and 30%/20% for RIFINs #1 and #5, respectively. The experiment was performed once.
Extended Data Figure 7 | LILRB1-binding RIFIN did not bind to other LILRs. a, The sequence encoding the variable region of LILRB1+ RIFIN #1 was transfected into 293T cells, and the transfectants were stained with LILR–Fc fusion proteins. The levels of LILR–Fc binding are indicated as mean fluorescence intensities (MFIs). Control indicates fluorescence of cells reacted only with the secondary antibody. The experiment was performed once. b, c, Binding of LILR–Fc fusion proteins to IEs. RIFIN #1- and RIFIN #5-transgenic IEs were stained with 11 LILR–Fc fusion proteins and analysed using flow cytometry. Control indicates fluorescence of cells reacted only with the secondary antibody. The proportions of IEs stained with LILR–Fc fusion proteins are shown. The experiment in b was replicated twice and experiment in c was performed once.
Extended Data Figure 8 | Binding of wild-type and mutated LAIR1 to IEs. a, IEs from RIFIN-transgenic parasites (RIFIN #1, PF3D7_1254800; RIFIN #2, PF3D7_0223100; RIFIN #3, PF3D7_0500400; RIFIN #4, PF3D7_1000500; RIFIN #5, PF3D7_1254200; RIFIN #6, PF3D7_1400600; RIFIN #7, PF3D7_1040300; and RIFIN #8, PF3D7_1101100) were stained with wild-type LAIR1–Fc (wtLAIR1–Fc, red), mutated LAIR1–Fc (muLAIR1–Fc, blue), and control–Fc (shaded-grey histogram) fusion proteins. The experiments were replicated twice. b, LAIR1–Fc bound to erythrocytes infected with P. falciparum derived from Thai patients with malaria. Schizont-stage IEs derived from Thai patients with malaria were stained with LAIR1–Fc (vertical) and LILRB1–Fc (horizontal) fusion proteins, followed by Vybrant Green. Vybrant Green-positive cells were analysed. The percentages of LAIR1-ligand single-positive, LILRB1-ligand single-positive and LAIR- and LILRB1-ligand double-positive IEs are shown. The experiments were replicated twice.
Extended Data Figure 9 | Functional analysis of cells expressing LAIR1 and LILRB1. a–c, Erythrocytes infected with LAIR1–Fc-binding parasites (RIFIN #8 transgenic parasites (a), parasites from Thai patient 1 (b), LAIR1–Fc-binding parasites enriched by cell sorting from Thai patient isolate 4 (LAIR1-L enriched patient 4, c)) or erythrocytes infected with parasites that did not bind LAIR1–Fc (RIFIN #3 transgenic parasites (a), parasites from Thai malaria patient 3 (b, c)) were prepared (left). LAIR1 reporter cells were co-cultured with these IEs and expression levels in reporter cells were analysed using flow cytometry (right). Immobilized collagen IV served as a positive control for LAIR1 reporter activation. Proportions of GFP-expressing cells are shown as the mean ± s.d. (n = 3 biologically independent samples, one-way ANOVA with Tukey’s post hoc test). d, LILRB1 expressed by the NK cell line NKL. e, Flag-tagged RIFINs were expressed by K562 cells stably transfected with LILRB1 + RIFIN #1 or LILRB1 − RIFIN #5, f, RIFIN-expressing K562 cells or parental K562 cells were used as targets for NKL. Data represent the mean ± s.d. (n = 3 technically independent samples). *P < 0.05, two-way ANOVA with Tukey’s post hoc test. The experiments in d and e were replicated at least twice.
Extended Data Figure 10 | Age dependence of the antibody response to LILRB1⁺ and LILRB1⁻ RIFINs. Plasma IgG positivity for the recombinant proteins comprising the variable regions of LILRB1⁺ RIFIN #1 and LILRB1⁻ RIFIN #5 as well as GLURP_R2 in 222 Tanzanian individuals divided into age groups. Error bars represent 95% confidence interval. P values were calculated using logistic regression comparing percent responders among children aged 0–1 years to children of the other age groups.
Experimental design

1. Sample size
   Describe how sample size was determined.
   No statistical method was used to pre-specified sample size. Sample size was determined on the basis of control samples and the experimental samples. We have basically used the data from at least two or three independent experiments when necessary. The P values results from statistical analyses suggested that the samples sizes in our study are sufficient. With regard to binding assay, the sample size was small, because it is difficult to get the patient numbers needed. Still the P values results from statistical analyses suggested that the samples sizes in our study are sufficient, although an extended study is required to confirm the findings.

2. Data exclusions
   Describe any data exclusions.
   No data were excluded from the analyses.

3. Replication
   Describe whether the experimental findings were reliably reproduced.
   All attempts at replication were successful.

4. Randomization
   Describe how samples/organisms/participants were allocated into experimental groups.
   All samples were assigned to groups randomly.

5. Blinding
   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.
   The investigators were not blinded to allocation during data collection and analyses.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.
6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

| n/a | Confirmed |
|-----|-----------|
| ☑   | ☑         |

- The exact sample size ($n$) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
- A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- A statement indicating how many times each experiment was replicated
- The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
- A description of any assumptions or corrections, such as an adjustment for multiple comparisons
- The test results (e.g. $P$ values) given as exact values whenever possible and with confidence intervals noted
- A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
- Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

### Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.

- Statistical analyses were performed with the GraphPad Prism 7 software (GraphPad), the R software (the R Foundation) and Excel (Microsoft).
- Flow cytometric analyses were performed with the FlowJo analysis software (FlowJo).
- Electrophoretic analyses were performed with the Image Gauge (Millipore).
- Mass analyses were performed with the Mascot (Matrix Science).

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). Nature Methods guidance for providing algorithms and software for publication provides further information on this topic.

### Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

- No unique materials were used.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species). Antibodies were obtained from the following vendors: allophycocyanin (APC) conjugated anti-human IgG Fc antibody (109-136-098, Jackson ImmunoResearch Laboratories), phycoerythrin (PE)-conjugated anti-human IgG Fc antibody (109-116-098, Jackson ImmunoResearch Laboratories), anti-His antibody (clone 28-75; WAKO), anti-DYKDDDDK (FLAG) antibody (clone L5; Biolegend), APC conjugated anti-CD85j (LILRB1) antibody (clone HP-F1; eBioscience).
10. Eukaryotic cell lines
   a. State the source of each eukaryotic cell line used. Human embryonic kidney 293T and Chinese hamster ovary (CHO) cells were obtained from the RIKEN Cell Bank. NKL cells were a gift from Dr Lanier (University of California, San Francisco). K562 cells were obtained from Cell Resource Center for Biomedical Research, Institute of Development, Aging and Cancer, Tohoku University.
   b. Describe the method of cell line authentication used. 293T and CHO cells were authenticated by the RIKEN Cell Bank. NKL cells were described in Exp. Hematol. 1996 Feb;24(3):406-15. K562 cells were authenticated by Cell Resource Center for Biomedical Research, Institute of Development, Aging and Cancer, Tohoku University.
   c. Report whether the cell lines were tested for mycoplasma contamination. Each cell line was regularly tested for mycoplasma contamination by PCR.
   d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use. Not applicable.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals
    Provide details on animals and/or animal-derived materials used in the study. Not applicable, because our study does not include animal studies.

Policy information about studies involving human research participants

12. Description of human research participants
    Describe the covariate-relevant population characteristics of the human research participants. For blood samples from Thai patients, clinical P. falciparum isolates were collected from patients living in the Mae Sariang district, Mae Hong Son Province in Thailand. For binding assay, blood samples from children, aged 1 month to 5 years, were collected in Tanzania. For serological analyses, plasma samples from children and adults, aged 6 months to 60 years, were collected in Mkokola, Tanzania.
Flow Cytometry Reporting Summary

Form fields will expand as needed. Please do not leave fields blank.

Data presentation

For all flow cytometry data, confirm that:

1. The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
2. The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a ‘group’ is an analysis of identical markers).
3. All plots are contour plots with outliers or pseudocolor plots.
4. A numerical value for number of cells or percentage (with statistics) is provided.

Methodological details

5. Describe the sample preparation.
   The parasites (3D7 line and Thai patient) in culture media [10% O-type human serum in RPMI 1640] were mixed with new human erythrocytes and cultured in 10 cm dish (hematocrit: 6%) at 37°C under gas conditions of 5% CO2, 5% O2 and 90% N2. Infected red blood cells at schizont stage were obtained by 70% percoll density gradient centrifugation.
   Human peripheral blood mono-nucleated cells (PBMC) of healthy donors were isolated from fresh blood samples by Ficoll-Paque (Leucosep).

6. Identify the instrument used for data collection.
   FACSCalibur HG, FACS Verse (BD Biosciences)

7. Describe the software used to collect and analyze the flow cytometry data.
   Data collection: CellQuest pro, FACSuite (BD Biosciences)
   Data analysis: FlowJo (FlowJo)

8. Describe the abundance of the relevant cell populations within post-sort fractions.
   Sorted samples were > 63% LAIR1-Ligand positive (Extended data Figure 9c)

9. Describe the gating strategy used.
   293T cells and human PBMC were gated on FSC/ SSC- area, live(PI negative; cell line), transfected cells (GFP high; Figure 2c,2d, Extended data Figure 6a,7a).
   Red blood cells were gated on FSC/ SSC- area, nuclear staining(Vybrant Green positive; infected red blood cells), exclusion of non specific staining (anti-human IgGFc-PE negative; Extended data Figure 1)

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information. ☑
Corrigendum: Immune evasion of *Plasmodium falciparum* by RIFIN via inhibitory receptors

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*Nature* **552**, 101–105 (2017); doi:10.1038/nature24994

In Extended Data Fig. 1 of this Letter, the flow cytometry data panel showing uninfected erythrocytes stained with LILRB5-Fc was inadvertently duplicated from the panel showing uninfected erythrocytes stained with LILRB4-Fc. This figure has been corrected online, and the original incorrect panel is provided as Supplementary Information to this Corrigendum, for transparency. This error does not alter the results or conclusions of the Letter. We would like to thank the readers who brought this error to our attention. The original Letter has been corrected online.

Supplementary Information is available in the online version of this Corrigendum.