Supplementary Materials for

A Forward Genetic Screen Identifies Erythrocyte CD55 as Essential for *Plasmodium falciparum* Invasion

Elizabeth S. Egan, Rays H.Y. Jiang, Mischka A. Moechtar, Natasha S. Barteneva, Michael P. Weekes, Luis V. Nobre, Steven P. Gygi, Joao A. Paulo, Charles Frantzreb, Yoshihiko Tani, Junko Takahashi, Seishi Watanabe, Jonathan Goldberg, Aditya S. Paul, Carlo Brugnara, David E. Root, Roger C. Wiegand, John G. Doench and Manoj T. Duraisingh*

correspondence to: mduraisi@hsph.harvard.edu

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Other Supplementary Materials for this manuscript includes the following:

Supplementary database as zipped archive:
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Dataset S2. RIGER analysis results for pooled shRNA screen of erythrocyte proteome
Dataset S3. Interactome data
Dataset S4. Phenotypes and expression patterns of erythropoiesis candidates
Dataset S5. RIGER analysis for pooled shRNA screen of human blood group genes

Materials and Methods

shRNA library design

Genes for the erythrocyte proteome shRNA library primarily came from the proteome described by Pasini *et al.* (*12*). In addition to these ~600 genes, we included approximately 100 genes highly expressed in the reticulocyte transcriptome (*38*) and modifying enzymes such as glycosyltransferases and sulfotransferases, for a total of 862 genes (Supplemental Dataset S1). A pooled library of 5530 TRC shRNA clones was produced from the sequence-validated TRC shRNA library (*39*) and included shRNAs targeting control genes such as luciferase. All genes were targeted by a minimum of 5 shRNAs. For the blood group-focused screen, we produced a different pooled library consisting of 308 TRC shRNA clones that target 42 human blood group genes. Each gene was targeted by at least 5 shRNAs.

Pooled shRNA screening

For the erythropoiesis screen, $CD34^+$ hematopoietic stem cells (HSC) from a single Caucasian bone marrow donor were obtained commercially (Lonza), and induced to proliferate and differentiate according to a published protocol with minor modifications (*8*). For each replicate, $3x10^5$ cells were initially grown in Iscove Modified Dulbecco-based medium (Biochrom) supplemented with FBS (Lonza), stem cell factor (SCF, R&D Systems), hydrocortisone (Invitrogen), IL-3 (R&D Systems), and erythropoietin (Amgen). On day 6, cells were transduced with the pooled lentivirus shRNA library (0.8ml of virus per $1x10^6$ cells) by spinoculation at 1000xg for 2 hours in 4 μg/ml polybrene. Transduced cells were selected with 2 μg/ml puromycin from days 7-11. Hydrocortisone and IL-3 were removed on day 8, and SCF was removed on day 11. On day 19, DNA was isolated from the orthochromatic erythroblasts and shRNA provirus sequences were quantified using Illumina sequencing. Six independent experiments were performed. For the screen of blood group genes, orthochromatic erythroblasts were generated in a similar manner, except a modified medium with a higher concentration of holotransferrin and 5% pooled solvent/detergent treated human plasma (Octaplas, Octapharma) instead of FBS was used in the final two replicates to improve cell yields (*9*). On day 17, orthochromatic erythroblasts were infected with schizont stage *P. falciparum* strain $3D7^{attB}$/GFP

(*40*) that had been isolated by magnetic purification using a MACS Magnet (Miltenyi), at 4-8% parasitemia. Uninfected knockdown cRBCs were maintained in parallel as a control. After 18 hours, the cells were gently fixed in PBS/0.1% paraformaldehyde for 10 minutes, and GFP-positive, parasitized cRBCs were sorted in a $BL2^{+}$ FACSAria II flow cytometer (BD Biosciences). Genomic DNA was isolated from parasitized and control cells, and hairpin sequences were quantified using Illumina DNA sequencing.

Bioinformatic methods

We applied the RNAi Gene Enrichment Ranking (RIGER) algorithm to evaluate candidate genes from the pooled shRNA screens, similar to the method described by Luo *et al.* (*15*). This method is nonparametric and calculates gene scores from a collection of shRNA phenotype profiles. For the erythroid development screen, shRNAs were first scored according to their measured effect on cRBC development based on the number of sequence reads in the pool of day 19 cRBCs relative to the original shRNA library, and a raw enrichment score was calculated. Secondly, a Normalized Enrichment score (NES) was derived by correcting for different numbers of shRNAs used in different genes. This was done by normalizing against the directional mean of a null distribution generated by 10,000 random permutations. shRNAs with low abundance in the library at baseline were not included in the analysis. To generate a robust candidate gene list, we added additional criteria requiring individual shRNAs to have reproducible phenotypes in 5 out of 6 experimental replicates, and genes to have at least a third of shRNAs with a measurable phenotype.

The RIGER algorithm was also used to analyze sequencing data from the parasite infection screen. Three independent experiments were performed using bone marrow from a single donor. shRNAs were scored according to their measured effect on invasion of cRBCs by *P. falciparum* parasites based on the number of sequence reads for each hairpin in the GFP-positive, parasitized cells (collected by FACS) as compared to the control cRBCs that were not infected with parasites. Normalized Enrichment Scores were derived by correcting for different numbers of gene-specific shRNAs, as described above. Gene rankings were performed for each of the replicates. shRNAs with low representation in the non-parasitized control pool of cells were not included in the analysis.

For the erythroid gene expression analysis, the datasets published by Merryweather-Clarke *et al.* (*41*) were used. The time points represent harvest times 4, 5-6, 8-11, or 13-15 days in culture (populations of CFU-E, Pro-Es, Int-Es, and Late-Es, respectively). We performed k-means clustering of the erythropoiesis candidates and normalized array data from GeneChip Human Genome U133_Plus_2.0 were used. Mahalanobis distances were calculated for the cluster construction. We defined a total of 4 clusters of genes where shRNAs increase or decrease in relative abundance over the course of erythroid differentiation.

For the interactome analysis, we used protein-protein interaction data retrieved from the GeneMania database and filtered for only physical and predicted interactions. The interactome was constructed with Cytoscape 3.0.1 using the set of 116 erythropoiesis associated genes. The gene ontology data were used to classify the gene groups and spatially organize the interaction network.

Mouse gene knockout phenotypes listed in Supplemental Database S4 were classified by mining MGI dataset and searching for terms related to hematology, such as "abnormal hematopoiesis" (MP:0001600, MP:0000236, MP:0001604, MP:0001605, MP:0000241, MP:0002402). Any mouse phenotypes that are not hematology related were classified as 'other phenotypes'.

To assess gene-specific variation in populations with high or low-moderate ancestral exposure to malaria, genomic variation data from phase 1 of the 1000 Genomes Project (ftp://ftp-trace.ncbi.nih.gov/1000genomes/ftp/release/20110521/) were analyzed in the context of human genome version hg19 from UCSC (http://genome.ucsc.edu). Human populations and population membership from the 1000 Genomes project are given in ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/20131219.populations.tsv and ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/technical/working/20130606_sample_info/20130606_g1k.ped, respectively. Non-synonymous protein coding variants were called using the VariantAnnotation package of Bioconductor (http://www.bioconductor.org/packages/release/bioc/html/VariantAnnotation.html) with gene models from ENSEMBL+hg19(https://galaxy.cbio.mskcc.org/library/index).
The isoform used for CD55 was: ENST00000367064 (DAF-2 isoform). As positive and negative controls, we used HBB: ENST00000335295 (HBB-001); and PRKACG: ENST00000377276. The amino acid changes and rs numbers observed in CD55 are R52L,

rs28371588; L82R, rs147474393; Y133H, rs199922774; A227P, rs60822373; I231V, rs147483928; V333I, rs116473488; G354A, rs186602607; G372E, rs199553775. Populations were binned into groups with high or low current or ancestral exposure to malaria based on DALY (disability-adjusted life year per 100,000 population) values for malaria from The World Health Organization (http://www.who.int/entity/healthinfo/global_burden_disease/gbddeathdalycountryestimates2004.xls), where high exposure is set at DALY $\geq$ 10 and low at DALY < 10. Individuals with variation in CD55 were summed for each group. The statistical significance for variant occurrence was calculated using Fisher's exact test, with the null hypothesis that variation within populations with high or low-to-moderate exposure to malaria is the same.

Generation of cultured red blood cells with specific gene knockdowns

Individual knockdown cRBCS were produced from bone marrow-derived $CD34^+$ cells (Lonza) except for the erythropoiesis candidates shown in Fig. S3, for which $CD34^+$ cells mobilized from peripheral blood of GCSF-stimulated donors were used (obtained from the HSCI-BCH FACS Core). In all individual knockdown experiments, the human plasma-based media and associated protocol was used, as described for the blood group screen. Lentiviral transduction was performed on day 6, using individual virus preparations produced from the following TRC shRNA clones: EmpT: pLKO.1 without an shRNA sequence, KPNB1: TRCN0000290197; SLC12A4: TRCN0000042933; FECH: TRCN0000083654; BCL2L1: TRCN0000299586; CD44-1: TRCN0000308110; CD44-2: TRCN0000296191; CD55-1: TRCN0000057164; CD55-2: TRCN0000255375. Throughout development, growth and differentiation were monitored by hemocytometer-based assessment of cell counts as well as by light microscopy of cytospin preparations stained with May-Grünwald and Giemsa. After day 13, cRBCs were co-cultured on a murine stromal cell layer to maximize enucleation and remove cellular debris, as previously described (*8*). Cells were harvested once the population reached >50% enucleation, after a minimum of 19 days in culture. Prior to parasite invasion assays, residual nucleated cRBC precursors were removed by syringe filtration using 5μM Supor filters (Pall).

Flow cytometry-based detection of cell surface molecules

Expression of RBC surface proteins was measured in control and knockdown cRBCs by flow cytometry. $1x10^6$ cRBCs were washed twice with PBS/0.3% BSA and incubated in primary mouse monoclonal antibodies for one hour at 4 degrees Celsius. Antibodies used: CD44: BRIC 222 (1:10,000, IBGRL); CD55: BRIC-216-PE (1:50, IBGRL), GYPA: anti-GPA-FITC (1:50, Stem Cell Tech); GYPC: BRIC10-FITC (1:10, Sana Cruz); Basigin: anti-CD147 (clone MEM-M6/6, 1:1000, Ex-Bio); CR1: anti-CR1 (clone J3D3, 1:200, Santa Cruz); CD71: anti-CD71-PE (1:11, Miltenyi); Band3: BRIC6-FITC (1:50, IBGRL); CD49d: anti-CD49d-APC (1:11, Miltenyi); GYPB: anti-GPA/GPB (clone E3, 1:10,000, Sigma). For detection of GYPB, cells were first treated with trypsin to remove GYPA. When non-conjugated primary antibodies were used, cells were washed and subsequently incubated for one hour in secondary antibody: goat anti-mouse-Alexafluor488 (Invitrogen) or goat anti-mouse-PE (Santa Cruz) at 1:2000. After incubation, cells were washed twice in PBS/0.3% BSA and analyzed on a MACS Quant Flow cytometer (Miltenyi Biotec). Data were analyzed using FlowJo Software (Treestar). Degree of knockdown was calculated by the following equation: $100-[MFI_{KD\ cRBC}/MFI_{EMPT\ cRBC}] \times 100$, where MFI is the mean fluorescence intensity corrected for background.

P. falciparum **culture and invasion assays.**

The strains 3D7, W2mef, HB3, D10, 7G8, and T994 are standard laboratory-adapted strains of *P. falciparum*. 3D7ΔRH2b, 3D7ΔEBA175, W2mefΔEBA181, T994ΔRH1 and 3D7ΔEBA140 are laboratory-adapted strains with deletions of individual invasion ligands. Field strains Th82.09, Th032.09, Th079.09, and Th026.09 were isolated from malaria patients in Senegal. The CD55-null (Inab phenotype) cells used for PMP and other assays in the main text were obtained from a Japanese patient who presented with an ovarian cyst and has a 261 G>A nucleotide change in the second exon of CD55 which is predicted to change Trp87 to a stop codon (*29*). Control CD55-positive erythrocytes were from two normal Japanese donors. The second sample of Inab cells (Inab-2) used for Fig. S8 was from a 91 year old Japanese woman with a 508 C>T nucleotide change in exon 4 of CD55, which is predicted to change Arg170 to a stop codon (*31*).

P. falciparum parasites were routinely cultured in human O-positive peripheral blood erythrocytes in complete RPMI medium with 0.5% albumax at 2% hematocrit at 37 degrees

Celsius in 5% CO_2 and 1% oxygen. Parasite invasion assays were performed using synchronized late-stage schizont parasites that were isolated using a MACS magnet (Miltenyi). For invasion assays using cRBCs, purified parasites were added to fully differentiated, enucleated knockdown or control cRBCs at 0.3% hematocrit in complete RPMI, at an initial parasitemia of 1%. Assays were performed in triplicate in 96 well plates. Ring parasitemia was counted the following day using bright-field microscopy of cytospin preparations stained with May-Grünwald and Giemsa. At least 1000 cells were counted for each well. For invasion assays using peripheral blood erythrocytes, magnet-purified schizonts were added to acceptor erythrocytes at 0.5% hematocrit in complete RPMI, at an initial parasitemia of 1%. For clinical isolates, assays were performed in 0.25% albumax and 0.25% heat-inactivated human AB serum. Assays were plated in triplicate in 96 well plates. The following day, the cells were harvested and stained with 1:2000 SYBR Green I for 20 minutes at room temperature. After two washes, the cells were analyzed for DNA content in a MACS Quant cytometer to identify parasitized cells. Data were analyzed using FlowJo software (Treestar). Flow cytometry results were confirmed by analyzing corresponding thin blood smears.

Attachment Assay

P. falciparum strain 3D7 schizont stage parasites were isolated by magnet purification on a MACS magnet, washed, and incubated in complete RPMI with 2 μM Compound 2 to prevent late stage-schizont rupture (*42*). After several hours, schizonts were washed four times in complete RPMI to remove Compound 2 and allow egress, counted, and added to wt control or CD55-null RBCs in the presence or absence of 1 μM cytochalasin-D, which allows parasite attachment to the RBC surface but inhibits actin polymerization and parasite entry. Wells with 50 units/ml heparin, which interferes with early attachment, were used as a negative control. Assays were performed in 12 well plates under the following conditions: initial parasitemia 10%, hematocrit 0.5%, and assay volume 650μl. Aliquots were taken at the following timepoints: T0 (0 minutes), T1 (30 min), T2 (60 min), T3 (180 min) and fixed in 0.116M sucrose and 2% glutaraldehyde to crosslink any parasites attached to the cell surface. Samples were subsequently stained in 1:1000 SYBR Green I for 20 minutes, and then analyzed for DNA content in a MACS Quant cytometer to quantify the percent cells with attached or invaded parasites. Percent of

schizonts in each well was also followed over time. Data were analyzed using FlowJo software (Treestar), and normalized for percent attachment/invasion detected in presence of heparin.

Plasma membrane profiling

Plasma membrane profiling (PMP) was performed as described previously (*30*). Briefly, 1.3×10^8 of each cell type were washed with PBS. Surface sialic acid residues were oxidized with sodium meta-periodate (Thermo) then biotinylated with aminooxy-biotin (Biotium). The reaction was quenched, and the biotinylated cells incubated in a 1% Triton X-100 lysis buffer. Biotinylated glycoproteins were enriched with high affinity streptavidin agarose beads (Pierce) and washed extensively. Captured protein was denatured with DTT, alkylated with iodoacetamide (IAA, Sigma) and digested on-bead with trypsin (Promega) in 200 mM HEPES pH 8.5 for 3h. Tryptic peptides were collected and labelled using TMT reagents. The reaction was quenched with hydroxylamine, and TMT-labelled samples combined in a 1:1:1 ratio. Labelled peptides were enriched, desalted and separated into six fractions using tip-based strong cation exchange.

Mass spectrometry data was acquired and searched as previously described using an Orbitrap Fusion coupled with a Proxeon EASY-nLC 1000 LC pump (Thermo Fisher Scientific, San Jose, CA). Peptides were separated using a 2 hr gradient of 6 to 30% acetonitrile in 0.125% formic acid at a flow rate of 300 nL/min. Each analysis used a MultiNotch MS3-based TMT method. Mass spectra were processed using a Sequest-based in-house software pipeline. Data were searched using the human Uniprot database (April 2014) concatenated with common contaminants, and filtered to a final protein-level false discovery rate of 1%. Proteins were quantified by summing TMT reporter ion counts across all peptide-spectral matches using in-house software. For protein quantitation, reverse and contaminant proteins were removed, then each reporter ion channel was summed across all quantified proteins and normalized assuming equal protein loading across all samples. Gene Ontology Cellular Compartment terms were added and p-values (Significance A) adjusted with the Benjamini Hochberg method using Perseus version 1.4.1.3.

Supplementary Text

Pooled shRNA screening for host determinants of *P. falciparum* infection

In preliminary experiments, we attempted to screen the large erythrocyte proteome library for genes that impact invasion and/or growth of *P. falciparum*, but these experiments only yielded an average of 20-300 parasitized cRBCs/shRNA. Since the power of pooled shRNA screens is limited by the fold representation of each shRNA in the pool of screened cells (*14*), the low number of parasitized cells/shRNA resulted in an abundance of false-positive candidates. The reasons for the low number of cells/shRNA were multi-factorial, including the low natural parasitemia of *P. falciparum in vitro*, size of the shRNA library, inherent heterogeneity of RBC development *in vitro* and spectral overlap between cRBC autofluoresence and GFP expression by *P. falciparum* (making it necessary to be conservative when sorting for GFP-positive cells).

By screening a smaller shRNA library focused on the blood group genes, we were able to obtain >700 infected cRBCs/shRNA, thus enabling a more robust screen. Importantly, given the complexity of this pooled shRNA screening approach for host determinants of *P. falciparum* infection, candidates identified by deep sequencing still required additional validation in order to be considered hits. Future larger screens could be similarly performed in a batch format using combinations of smaller libraries, or alternatively by starting with a larger number of HSCs. In addition, use of parasites that express fluorescent proteins with distinct spectra from cRBC autofluoresence could be employed.

Fig. S1

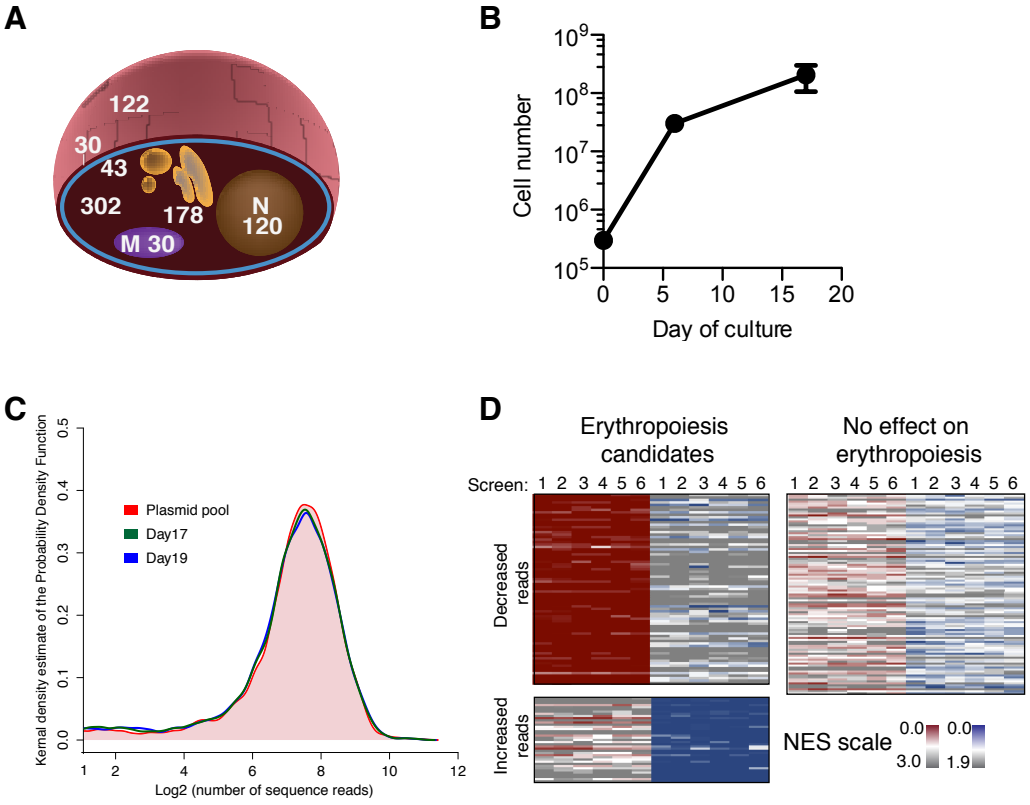

Fig. S1.

The erythroid proteome shRNA library targets 862genes expressed in the erythrocyte, and was used in a pooled shRNA screen. **(A)** Predicted localization of library components in a developing erythroid cell, showing 122 plasma membrane (red), 43 cytoskeletal (blue), 30 inner membrane-associated (dark red), 178 endomembrane (yellow), 30 mitochondrial (M), 120 nuclear-associated (N), and 302 cytoplasmic proteins. **(B)** Expansion of $CD34^+$ hematopoietic stem/progenitor cells during *ex-vivo* erythropoiesis. Cells were transduced with the erythrocyte proteome lentivirus shRNA library on day 6 and selected with puromycin from days 7-11. Dots and bars represent mean and standard deviation (SD), respectively (N=6 independent experiments). **(C)** Distribution of shRNA read abundance in erythrocyte proteome library prior to transduction (plasmid pool), in transduced cRBCs on day 17 of development (Day 17), or on day 19 of development (Day 19). shRNA proviruses were quantified by Illumina sequencing. Read abundance is presented as $\log_2$ (x-axis) and the distribution as the kernal density estimate of the probability density function (y-axis). **(D)** Heat map of RIGER analysis Normalized Enrichment Scores (NES) for 116 erythropoiesis candidates (left panels) and 100 control genes (right panel), in 6 independent replicates. Candidates were chosen in part for reproducibility. Horizontal rows represent data for individual genes. Red and blue scales reflect genes where shRNAs decreased or increased, respectively, in relative abundance from day 0 to day 19.

Fig. S2

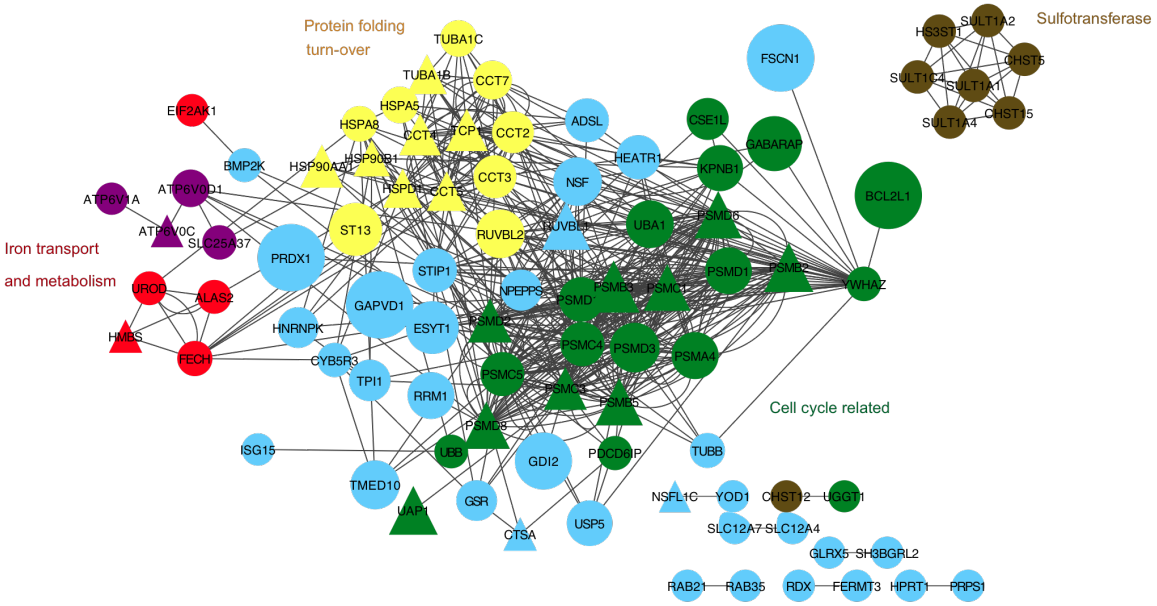

Fig. S2.

Interactome and functional categorization of erythropoiesis candidates according to known or predicted interactions. Circles represent genes in erythrocyte proteome library, whereas triangles represent genes not in the library. Size of symbols is proportional to neighborhood connectivity. The network is built from the set of 116 erythropoiesis associated genes. A connection is drawn if there is an interaction between an erythropoiesis gene (circles) and another erythropoiesis gene; or there is an interaction between an erythropoiesis gene and a gene with unknown role in erythropoiesis (triangles). All the gene-to-gene interactions have to be supported by the references listed in Dataset S3 such as documented physical or genetic interactions. The network highlights several biological pathways (with different color coding) that impact the erythropoiesis processes. The network also implies a possible role for the genes with unknown erythropoiesis functions (triangles) in the red blood cell developmental process.

Fig. S3

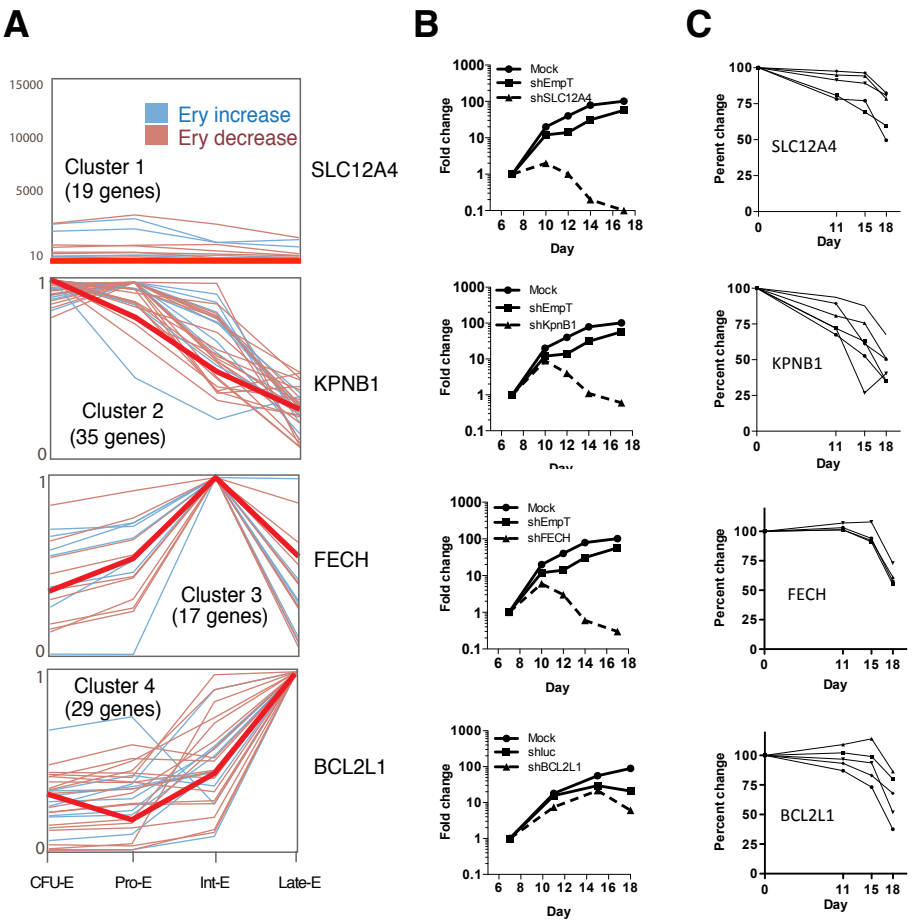

Fig. S3.

Expression of erythropoiesis candidate genes during erythroid development and validation of individual candidates. **(A)** Expression profiles of erythropoiesis candidate genes during *ex vivo* differentiation. Four clusters were defined by K-means clustering of the expression data published by Merryweather-Clarke *et al*. (*36*). Each cluster consists of genes where targeting shRNAs increased (blue) or decreased (red) in relative abundance over the course of erythroid differentiation. Expression profiles of SLC12A4, KPNB1, FECH, and BCL2L1 are highlighted in clusters 1, 2, 3 and 4, respectively. **(B)** Growth curves of developing cRBCs expressing shRNAs targeting erythropoiesis candidate genes (SLC12A4, KPNB1, FECH, and BCL2L1) or control cRBCs harboring control lentivirus (shEmpT). Cell expansion is expressed as fold change (y-axis) versus time (x-axis). Gene knockdown of >80% was measured by qPCR on day 12-13. **(C)** Change in abundance of all individual shRNAs targeting SLC12A4, KPNB1, FECH, or BCL2L1 within pooled library over the course of erythroid differentiation. Percent change in relative abundance as measured by Illumina sequencing is plotted (y-axis) versus time (x-axis).

Fig. S4

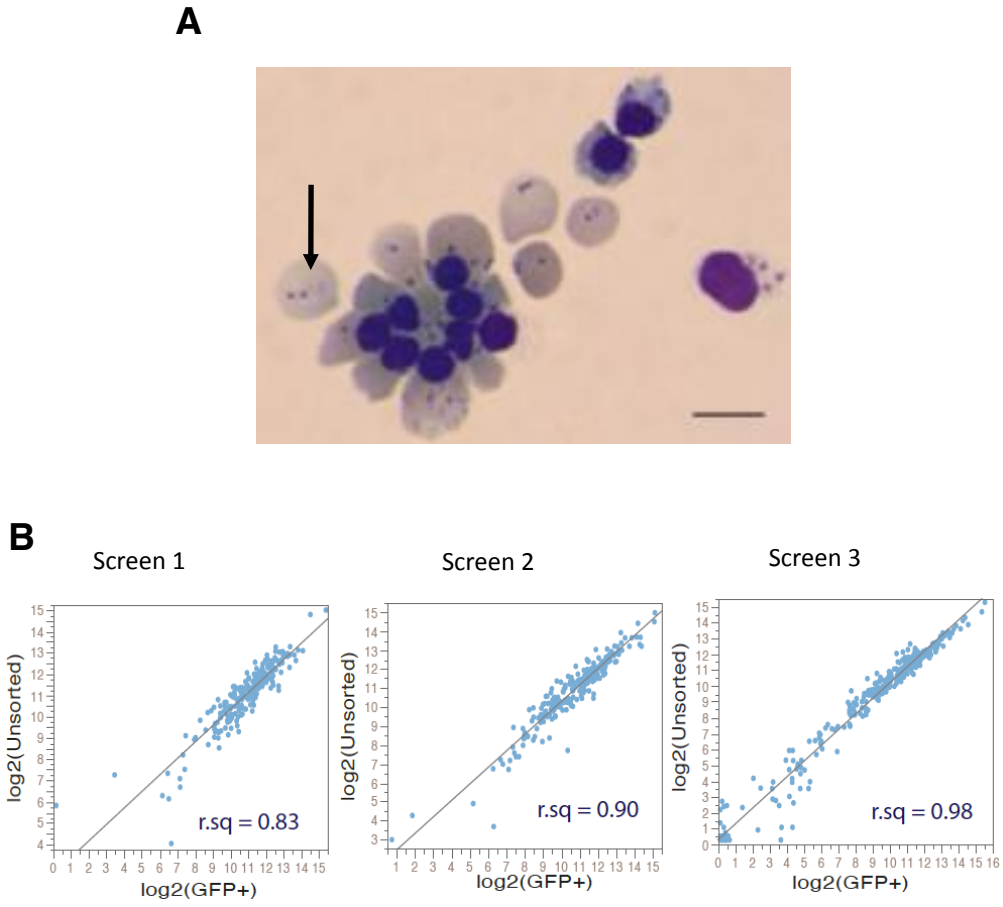

Fig. S4.

Screen for human blood group proteins that influence *P. falciparum* invasion of red blood cells. **(A)** Cytospin of knockdown cRBC 18 hours post-infection with GFP-expressing *P. falciparum* parasites. Cells were sorted via FACS based on GFP fluorescence, mounted on cytospin slides, and stained with May-Grunwald and Giemsa and visualized under 100x magnification. All cells from GFP-positive gate were noted to harbor ring stage parasites (arrow). Nuclei are stained purple. Scale bar measures 10 μm. **(B)** Scatter plots of shRNA sequencing reads from three replicates of the secondary screen of blood group genes. Data points represent individual shRNAs. Relative abundance of each shRNA in the parasitized cells is plotted as counts per million reads and expressed as $\log_2$ (x-axis), and abundance of each shRNA in unsorted control, non-parasitized cells is shown on y-axis.

Fig. S5

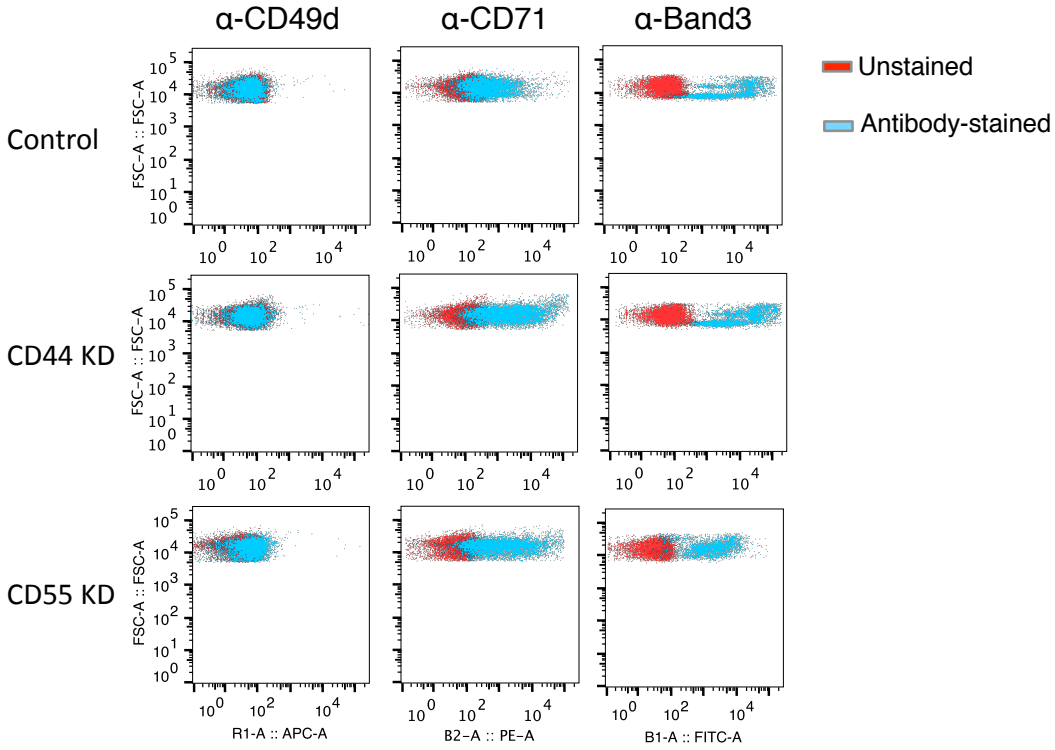

Fig. S5.

cRBCs with knockdown of CD44 or CD55 express age-appropriate amounts of CD49d, CD71, and Band3 on the cell surface. Day 19 cRBCs were analyzed by flow cytometry for surface expression of CD49d, CD71, and Band3 using monoclonal antibodies. Control cRBCs were transduced with shEmpT.

Fig. S6

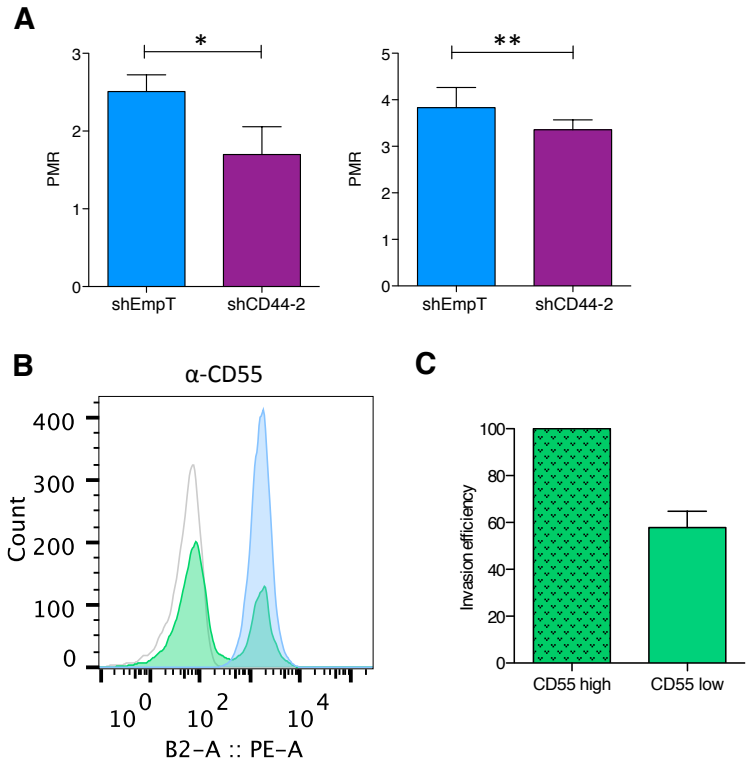

Fig. S6.

P. falciparum invasion assays using alternative shRNAs to deplete CD44 or CD55 in cRBCs. **(A)** Invasion of *P. falciparum* strain 3D7 into control cRBCs (shEmpT) or CD44-deficient cRBCs (shCD44-2). PMR= parasite multiplication rate from schizonts to rings. Mean ± SD. * p=0.02, ** p=0.08, one tailed t test. N=3. Two biological replicates are shown. **(B)** Histogram showing CD55 level on day 19 control cRBC transduced with shEmpT (blue) or CD55 knockdown cRBC expressing shCD55-2 (green). CD55 was detected by flow cytometry after staining with BRIC 216-PE. Unstained cells were used as a negative control for flow cytometry (grey). On day 19, two distinct populations were seen in the cells transduced with shCD55-2, thus enabling direct intra-well comparison of *P. falciparum* invasion into CD55-high and CD55-low cells. **(C)** Invasion efficiency of *P. falciparum* 3D7 into CD55-low day 19 cRBCs relative to CD55-high day 19 cRBCs. Assays were performed in triplicate using a mixed population of CD55- high (~30%) and CD55-low (~60%) of cells. Vybrant Dyecycle Violet (Life Technologies) was used to detect parasitemia and BRIC 216-PE was used to detect CD55 levels, both by flow cytometry. N= two biological replicates. Mean ± SD.

Fig. S7

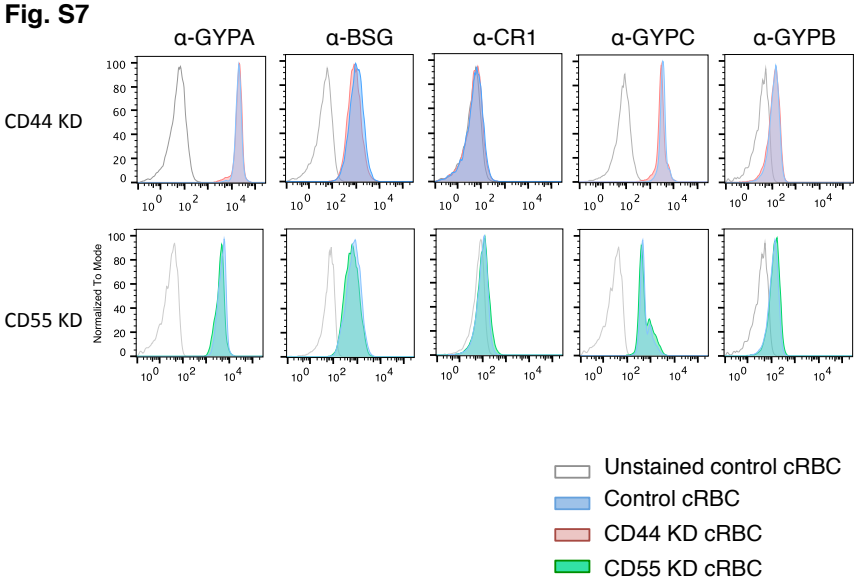

Fig. S7.

Levels of known *P. falciparum* receptors on CD44 and CD55 knockdown cRBCs as detected by flow cytometry using monoclonal antibodies. Blue= control cRBC, Red= CD44 knockdown cRBC, Green= CD55 KD cRBC, Grey= unstained control cRBC. Antibodies used are listed across the top. GYPA= glycophorin A, BSG= Basigin, CR1= CR1, GYPC= Glycopohorin C, GYPB= Glycophorin B. Cell numbers are normalized to mode (y-axis). Fluorescence units are on $\log_{10}$ scale (x-axis).

Fig. S8

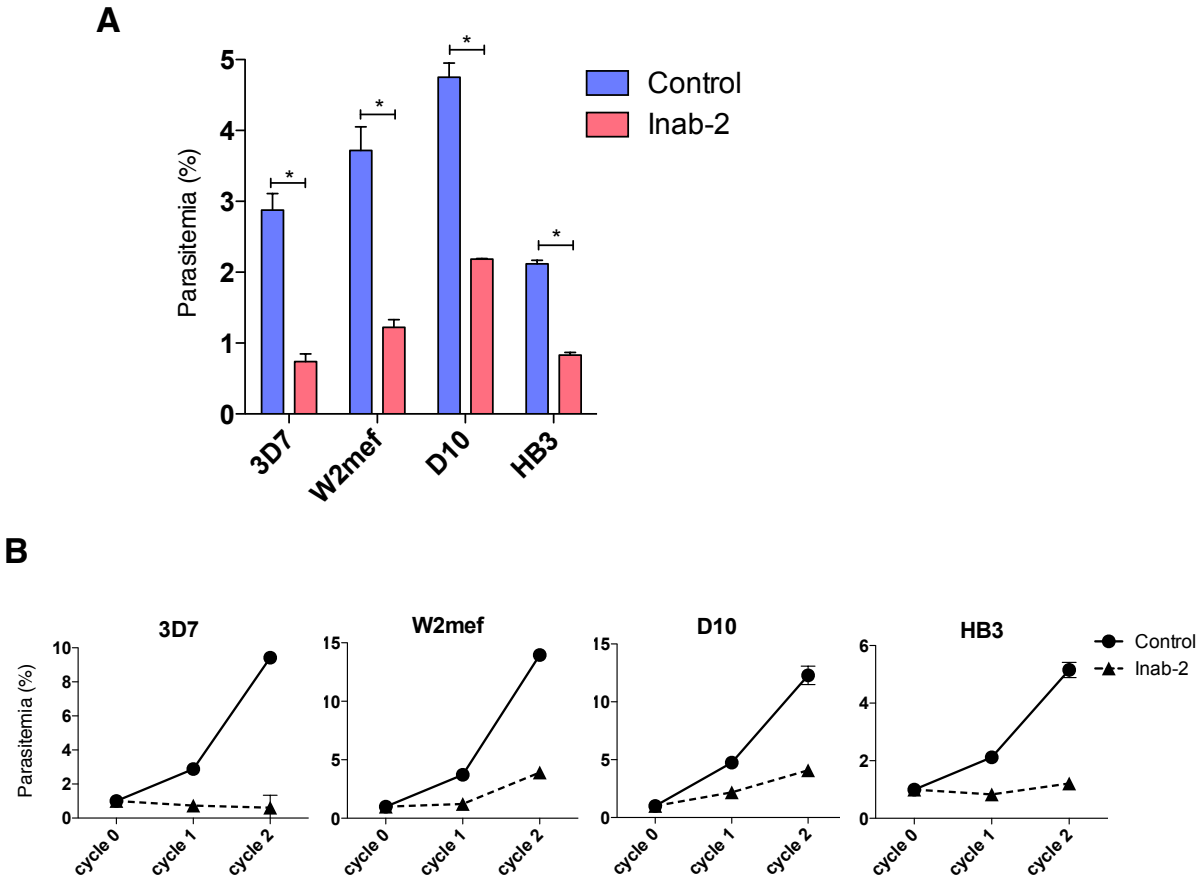

Fig. S8.

P. falciparum invasion and proliferation are impaired in RBCs from a second Inab donor (Inab-2). **(A)** Parasitemia of four laboratory-adapted *P. falciparum* strains 18 hours post-infection of wt control RBCs (purple) or Inab-2 RBCs (pink). *, $p<0.002$, two-tailed *t* test. **(B)** Parasite proliferation over the course of 72 hours, as measured by flow cytometry. Solid line with circles, control cells. Dashed line with triangles, Inab-2 cells. Mean ± SD, N=2 or 3.

Fig. S9

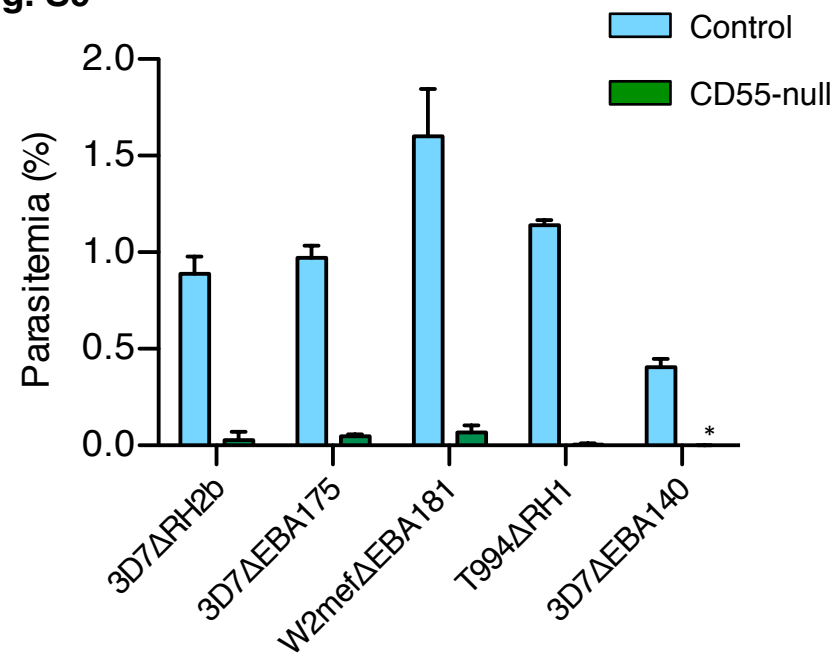

Fig. S9.

Erythrocyte CD55 is required for invasion by *P. falciparum* strains with deletions of individual invasion ligands. Parasitemia of laboratory-adapted *P. falciparum* strains 18 hours post-infection of control (blue) or CD55-null (green) erythrocytes. Parasitemia was quantified by flow cytometry (10,000 cells/well) and confirmed by blood smears. Mean ± SD, N=3. *, below limit of detection.

Fig. S10

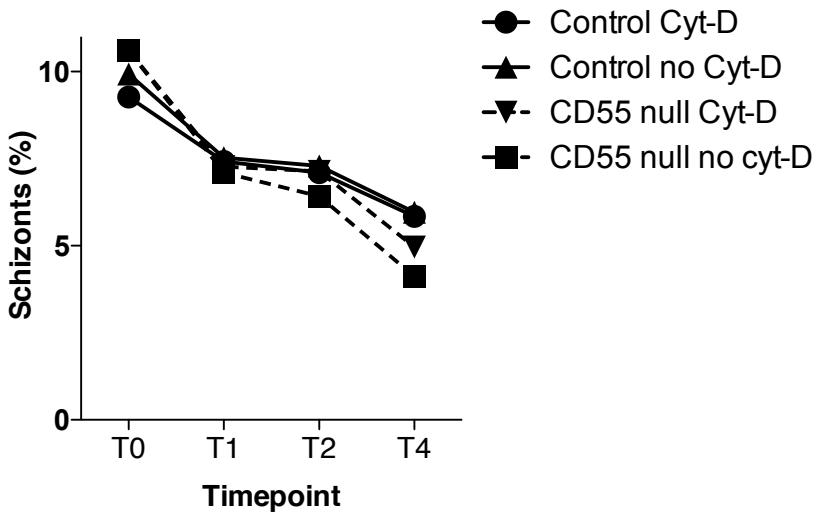

Fig. S10.

Rupture of schizont-stage parasites over time course of attachment assay. *P. falciparum* strain 3D7 schizonts were synchronized using magnet purification followed by Compound 2, washed, and added to control or CD55-null cells in the presence or absence of cytochalasin D (Cyt-D) at T=0. The percent schizonts was measured over time by flow cytometry, and is shown for a representative experiment. T1= 30min, T2=60 min, T3=180 min. 20,000 cells scored per well.

Additional Database (separate file)

Dataset S1. Gene list for erythrocyte proteome shRNA library. List of Gene symbols and Entrez Gene ID Numbers for genes included in the erythrocyte proteome shRNA library. Genes that were also included in the blood group secondary pool are indicated in bold type.

Dataset S2. RIGER analysis results for pooled shRNA screen of erythrocyte proteome. Erythropoiesis phenotypes associated with gene knockdown included a decrease in shRNA abundance on day 19 (decrease) or an increase in shRNA abundance on day 19 (increase) relative to the original library pool. RIGER analysis for each of 6 replicates was performed with weighted sum scores. Gene rank and Normalized Enrichment Scores (NES) are shown for each gene for each of 6 replicates.

Dataset S3. Interactome data. Pairwise interaction data and references are listed, in support of Figure S2.

Dataset S4. Phenotypes and expression patterns of erythropoiesis candidates. Data are presented for 116 hits. Expression data correspond to Fig. S3A. Gene Ontology (GO) annotation, gene description, and described mouse knockout phenotypes are listed.

Dataset S5. RIGER analysis for pooled shRNA screen of human blood group genes. RIGER analysis for each of 3 replicates was performed with weighted sum scores to identify candidate host factors for *P. falciparum*. Hairpin rank numbers, NES values gene rank numbers, and p-values are shown for each gene.