

Supplementary Information

***Plasmodium vivax* ligand-receptor interaction: PvAMA-1 domain I contains the minimal regions for specific interaction with CD71⁺ reticulocytes**

Gabriela Arévalo-Pinzón ^{1,2,+}, Maritza Bermúdez ^{1,3,+}, Diana Hernández ¹, Hernando Curtidor ^{1,4}, Manuel Alfonso Patarroyo ^{1,4*}

¹ Fundación Instituto de Inmunología de Colombia (FIDIC), Carrera 50 # 26-20, Bogotá, Colombia.

² PhD Program in Biomedical and Biological Sciences, Universidad del Rosario, Carrera 24 # 63C-69, Bogotá, Colombia.

³ MSc Program in Biological Sciences, Pontificia Universidad Javeriana, Carrera 7 # 40-62, Bogotá, Colombia.

⁴ School of Medicine and Health Sciences, Universidad del Rosario, Carrera 24 # 63C-69, Bogotá, Colombia.

+Equal contributors

Supplementary Table S1. A list of the primers used for amplifying PvAMA-1 fragments. The restriction sites for each one are highlighted in bold.

| Recombinant fragments | Primers | Used | Sequence | |
|-----------------------|-----------------------|--|---|---|
| PvAMA1 | Ectodo 1 | Heterologous Expression (<i>E. coli</i>) | 5'-CGG GAT CCC CTA CCG TTG AGA GAA GCA-3' | |
| | Ectodo 2 | | 5'-ACG CGT CGA CTA GTA GCA TCT GCT TGT TCG-3' | |
| PvDI-II | Ectodo 1 | | 5'-CGG GAT CCC CTA CCG TTG AGA GAA GCA-3' | |
| | DI-DIIR | | 5'-ACG CGT CGAC TTA CTC CAG GTC TAC TTC TTG-3' | |
| PvDII-DIII | DII-DIIIF | | 5'-CGG GAT CCC GTA AAA ATT TAG GAA ACG CC-3' | |
| | Ectodo 2 | | 5'-ACG CGT CGA CTA GTA GCA TCT GCT TGT TCG-3' | |
| PvAMA1 | AMA-1Cos-7F | | Expression on COS7 cells | 5'-GAA TTT AAA CCTA CCG TTG AGA GAA GC-3' |
| | AMA-1Cos-7R | | | 5'-GAT GGG CCC TAG TAG CAT CTG CTT GTT CG-3' |
| PvDI-II | AMA-1Cos-7F | 5'-GAA TTT AAA CCTA CCG TTG AGA GAA GC-3' | | |
| | AMA-1Cos-7EctoI-IIR | 5'-GCA GGG CCC CTC CAG GTC TAC TTC TTG-3' | | |
| PvDII-DIII | AMA-1Cos-7EctoII-IIIF | 5'-AGC CAG CTG GTG CGT AAT GAT TGG GAT AAA-3' | | |
| | AMA-1cos-7R | 5'-GAT GGG CCC TAG TAG CAT CTG CTT GTT CG-3' | | |

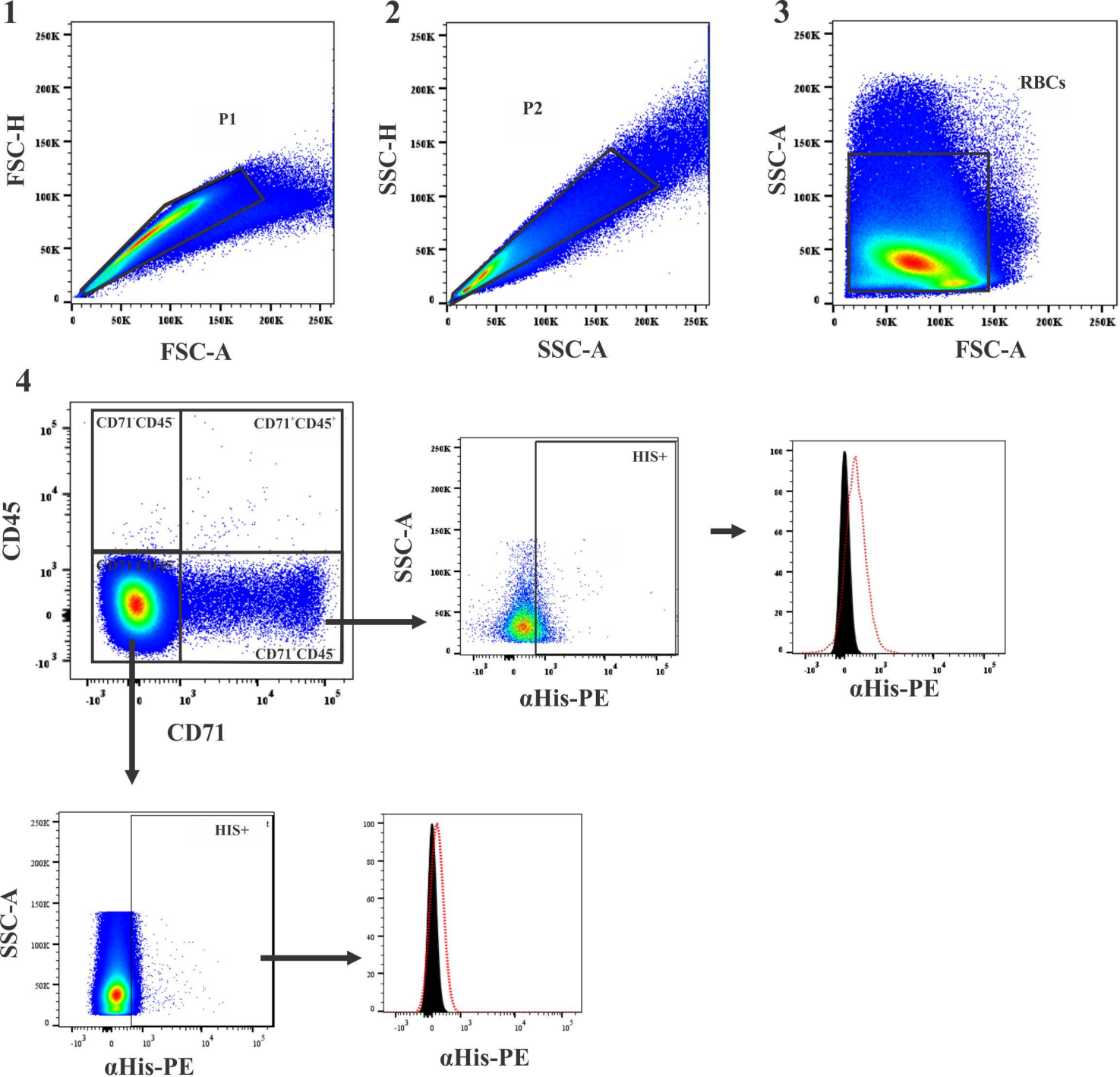


Figure S1. Flow chart used for selecting the study population by flow cytometry. Gating the general population regarding acquired events (P1, P2) for selecting RBC. Data was analyzed regarding CD45 labeling compared to CD71. Recombinant binding was compared to that of PE conjugated anti-histidine antibody binding to CD71⁺CD45⁻ (reticulocytes) or CD71⁻CD45⁻ cells (normocytes and older reticulocytes).

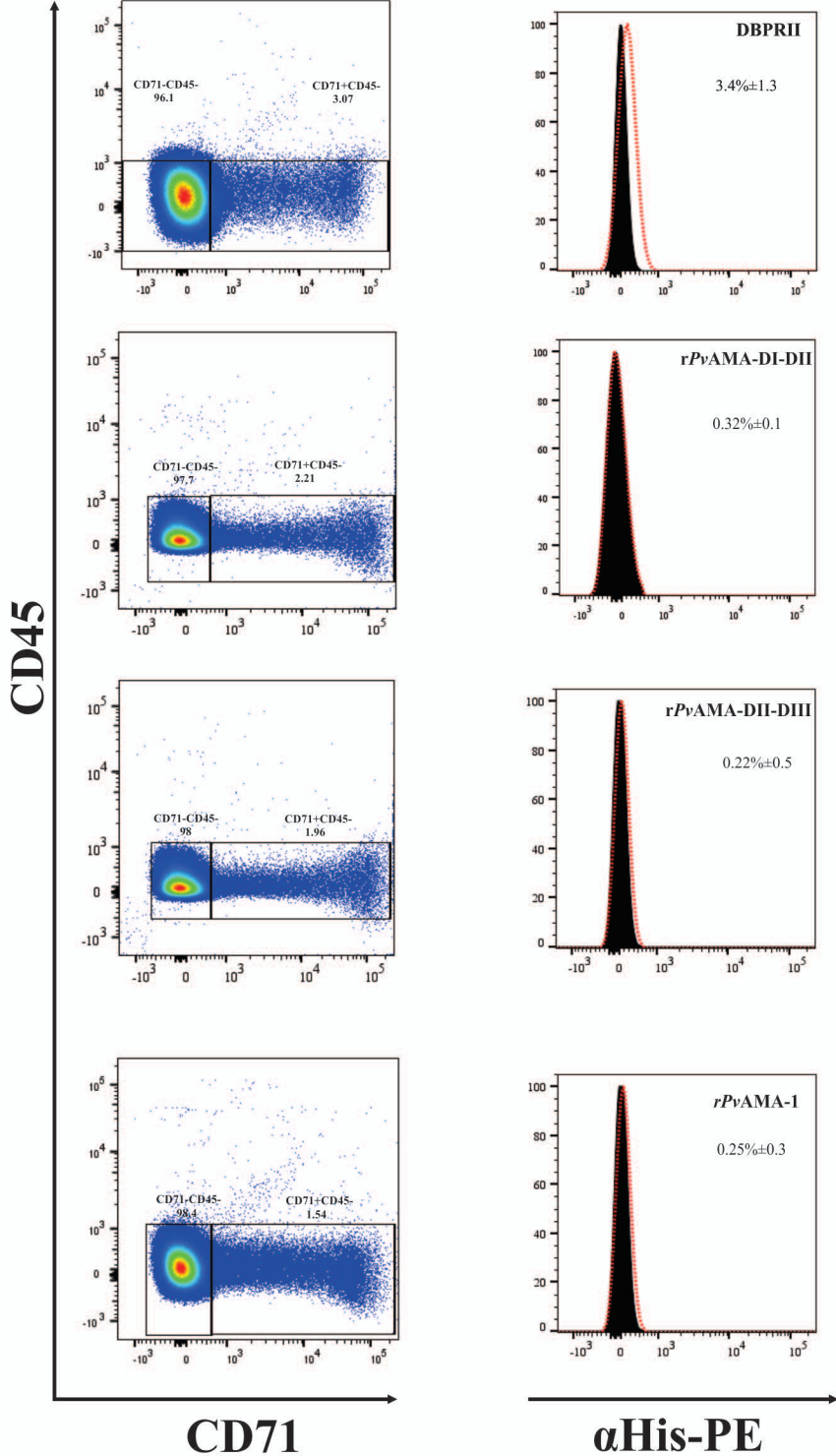


Figure S2. Flow cytometry CD71-CD45⁻ cell binding experiments. Binding to normocytes was determined by gating the CD71-CD45⁻ population. CD71+CD45⁻ cell binding percentages for each protein are shown on the X axis.


```

FJ784958.1 : TKPTCLINDKNFIATTALSHPEVDLDRDFPCSIYKDEIEREIK : 400
FJ784947.1 : TKPTCLINDKNFIATTALSHPEVDLEFPCSIYKDEIEREIK : 400
FJ784948.1 : TKPTCLINDKNFIATTALSHPEVDLEFPCSIYKDEIEREIK : 400
FJ785023.1 : TKPTCLINDKNFIATTALSHPEVDLDRDFPCSIYKDEIEREIK : 400
FJ785010.1 : TKPTCLINDKNFIATTALSHPEVDLDRDFPCSIYKDEIEREIK : 400
FJ785006.1 : TKPTCLINDKNFIATTALSHPEVDLDRDFPCSIYKDEIEREIK : 400
FJ784996.1 : TKPTCLINDKNFIATTALSHPEVDLDRDFPCSIYKDEIEREIK : 400
FJ784993.1 : TKPTCLINDKNFIATTALSHPEVDLDRDFPCSIYKDEIEREIK : 400
FJ785007.1 : TKPTCLINDKNFIATTALSHPEVDLDRDFPCSIYKDEIEREIK : 400
FJ784914.1 : TKPTCLINDKNFIATTALSHPEVDLDRDFPCSIYKDEIEREIK : 400
FJ784896.1 : TKPTCLINDKNFIATTALSHPEVDLDRDFPCSIYKDEIEREIK : 400
FJ784934.1 : TKPTCLINDKNFIATTALSHPEVDLDRDFPCSIYKDEIEREIK : 400
FJ784926.1 : TKPTCLINDKNFIATTALSHPEVDLDRDFPCSIYKDEIEREIK : 400
FJ785118.1 : TKPTCLINDKNFIATTALSHPEVDLDRDFPCSIYKDEIEREIK : 400
FJ785116.1 : TKPTCLINDKNFIATTALSHPEVDLDRDFPCSIYKDEIEREIK : 400
FJ785114.1 : TKPTCLINDKNFIATTALSHPEVDLDRDFPCSIYKDEIEREIK : 400
FJ785109.1 : TKPTCLINDKNFIATTALSHPEVDLDRDFPCSIYKDEIEREIK : 400
FJ785106.1 : TKPTCLINDKNFIATTALSHPEVDLDRDFPCSIYKDEIEREIK : 400
FJ784990.1 : TKPTCLINDKNFIATTALSHPEVDLEFPCSIYKDEIEREIK : 400
FJ784987.1 : TKPTCLINDKNFIATTALSHPEVDLEFPCSIYKDEIEREIK : 400
FJ784986.1 : TKPTCLINDKNFIATTALSHPEVDLEFPCSIYKDEIEREIK : 400
FJ784984.1 : TKPTCLINDKNFIATTALSHPEVDLEFPCSIYKDEIEREIK : 400
FJ784970.1 : TKPTCLINDKNFIATTALSHPEVDLEFPCSIYKDEIEREIK : 400
FJ784925.1 : TKPTCLINDKNFIATTALSHPEVDLDRDFPCSIYKDEIEREIK : 400
FJ784928.1 : TKPTCLINDKNFIATTALSHPEVDLEFPCSIYKDEIEREIK : 400
FJ785014.1 : TKPTCLINDKNFIATTALSHPEVDLEFPCSIYKDEIEREIK : 400
EU395595.1 : TKPTCLINDKNFIATTALSHPEVDLDRDFPCSIYKDEIEREIK : 400
EU395600.1 : TKPTCLINDKNFIATTALSHPEVDLDRDFPCSIYKDEIEREIK : 400
FJ784917.1 : TKPTCLINDKNFIATTALSHPEVDLEFPCSIYKDEIEREIK : 400
FJ784953.1 : TKPTCLINDKNFIATTALSHPEVDLDRDFPCSIYKDEIEREIK : 400
FJ784979.1 : TKPTCLINDKNFIATTALSHPEVDLDRDFPCSIYKDEIEREIK : 400
FJ784967.1 : TKPTCLINDKNFIATTALSHPEVDLDRDFPCSIYKDEIEREIK : 400
FJ784964.1 : TKPTCLINDKNFIATTALSHPEVDLDRDFPCSIYKDEIEREIK : 400
FJ784936.1 : TKPTCLINDKNFIATTALSHPEVDLDRDFPCSIYKDEIEREIK : 400
FJ784954.1 : TKPTCLINDKNFIATTALSHPEVDLDRDFPCSIYKDEIEREIK : 400

```

Figure S3. Aligning *Pv*AMA-1 domain I and domain II amino acids. The Sal-1 strain (PVX_092275) was taken as reference. Black highlights residues which were conserved in all the strains' and clinical isolates' sequences. The position and sequence of each synthetic peptide evaluated in inhibition and specific binding assays are shown. The yellow boxes show the variable peptides and the green ones the conserved peptides.

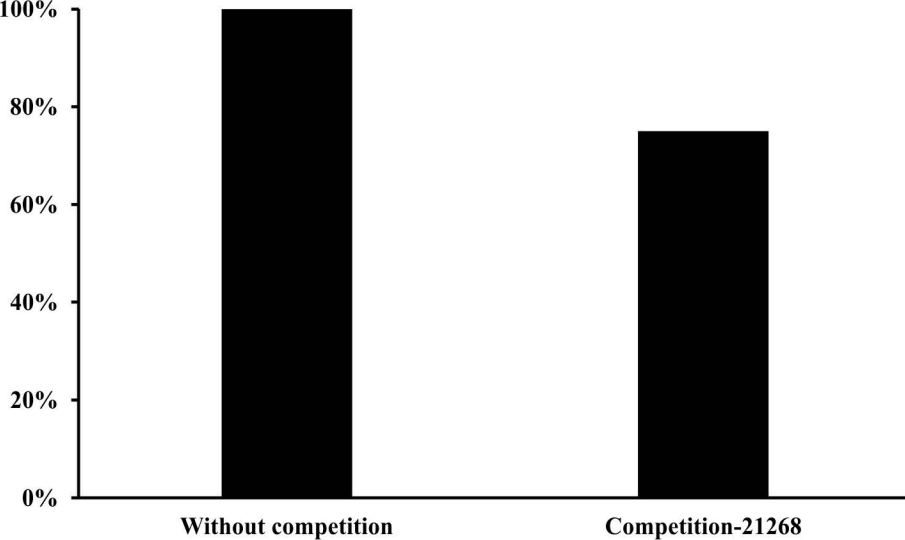


Figure S4. Rosette formation inhibition assay with peptide 21268. The values shown are the average relative percentages for *Pv*AMA-DI-II binding to UCB RBC in the absence (100%) or presence of peptide 21268. Standard deviations were below 5%.