

Supplemental Material

Potent AMA1-specific human monoclonal antibody against *Plasmodium vivax* Pre-erythrocytic and Blood Stages

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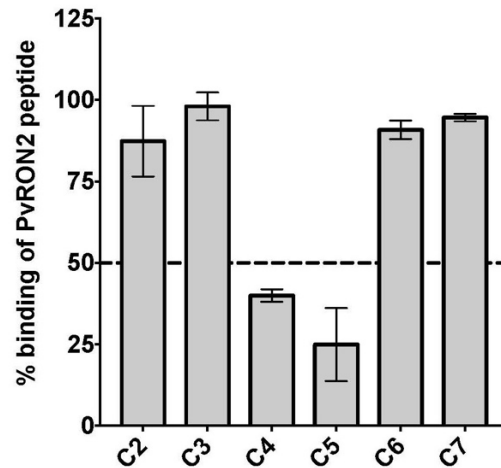
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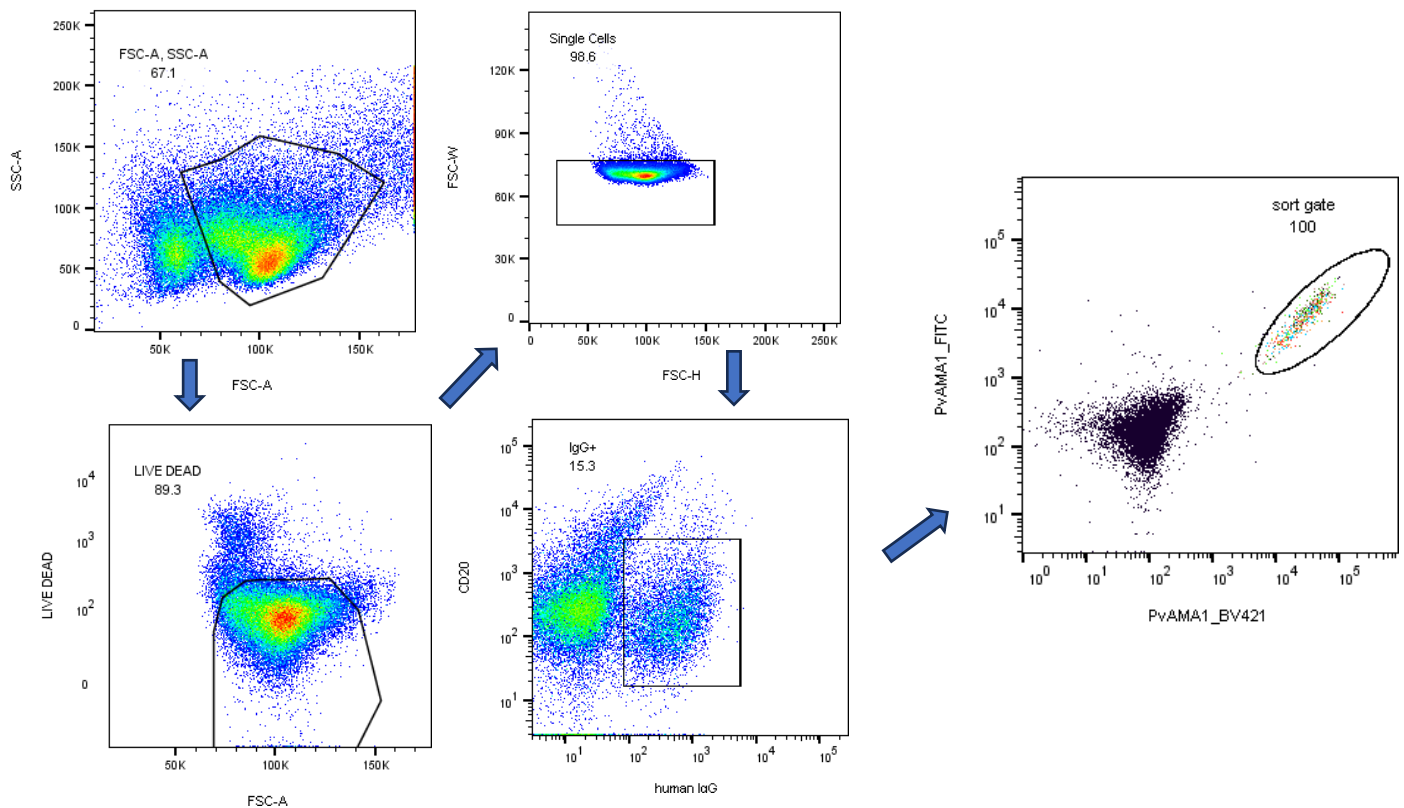
Supplemental Figure 1 – Blocking activity of serum from Cambodian individuals

Inhibition of PvRON2 binding to PvAMA1 by plasma samples from Cambodian donors. Plasma samples were tested at 1/50 dilution for the ability to inhibit binding of the PvRON2 loop to PvAMA1 in a plate-based assay. Samples were tested in duplicate. Data show mean and range; the dotted line shows 50% inhibition. PBMCs from donor C5 were selected for sorting B cells specific for PvAMA1 and subsequent MAb generation. Error bars show +/- SEM.



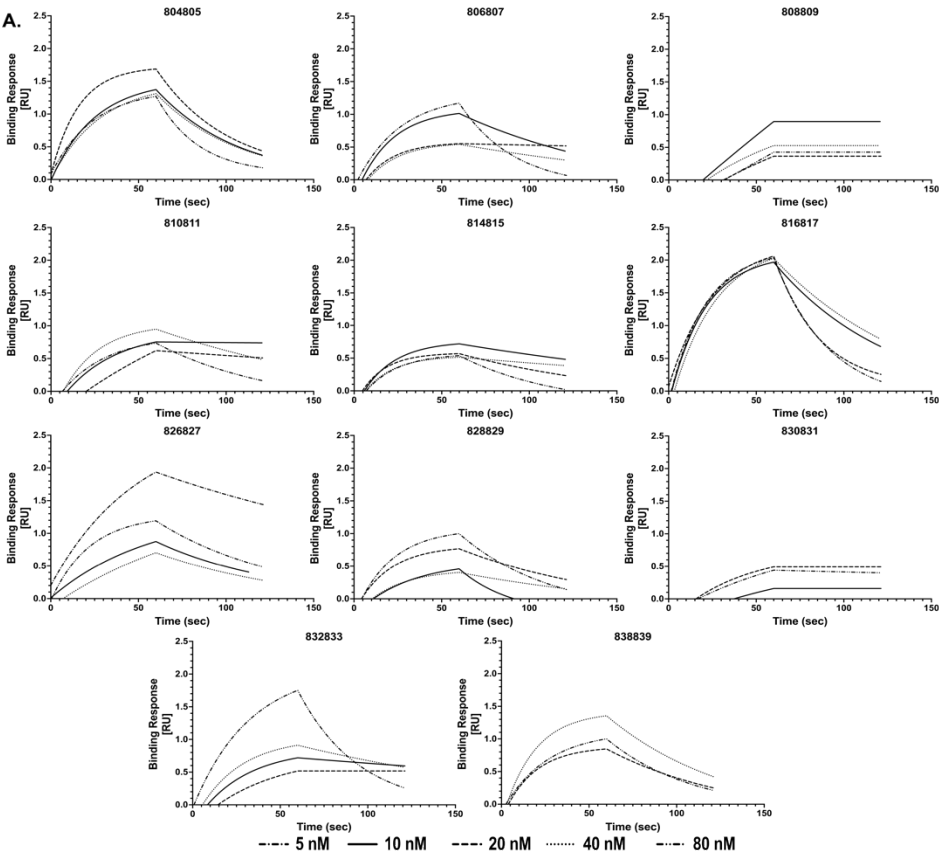
Supplemental Figure 2. Representative Flow diagram depicting PvAMA1-specific B cells that were isolated.

B cells were enriched using immunomagnetic positive selection with anti-CD19 magnetic MACS beads (Miltenyi Biotec, upper left panel). SYTOX Green Dead Cell Stain (Invitrogen) was used to gate out dead cells (lower left panel). Doublet discrimination was performed to exclude aggregated cells (upper middle panel) and stained with mouse anti-human CD20 (PE-Cy5.5; Invitrogen) and anti-human IgG Abs (PE-Cy7 clone G18-145; Becton Dickinson) to identify IgG expressing B cell (lower middle panel). Pv AMA1-specific B cells were identified using biotinylated PvAMA1 using Streptavidin coupled with Fluorescein isothiocyanate (FITC) or Brilliant Violet 421 (right panel showing sort gate). PvAMA1-cells were sorted on a BD FACSaria II.



Supplement Figure 3 – HumAb Single Cycle Kinetics Curves and Results

A) Binding response curves to various concentrations of PvAMA1 for each humAb. **B)** k_{on} and k_{off} rates for each humAb determined using SPR.

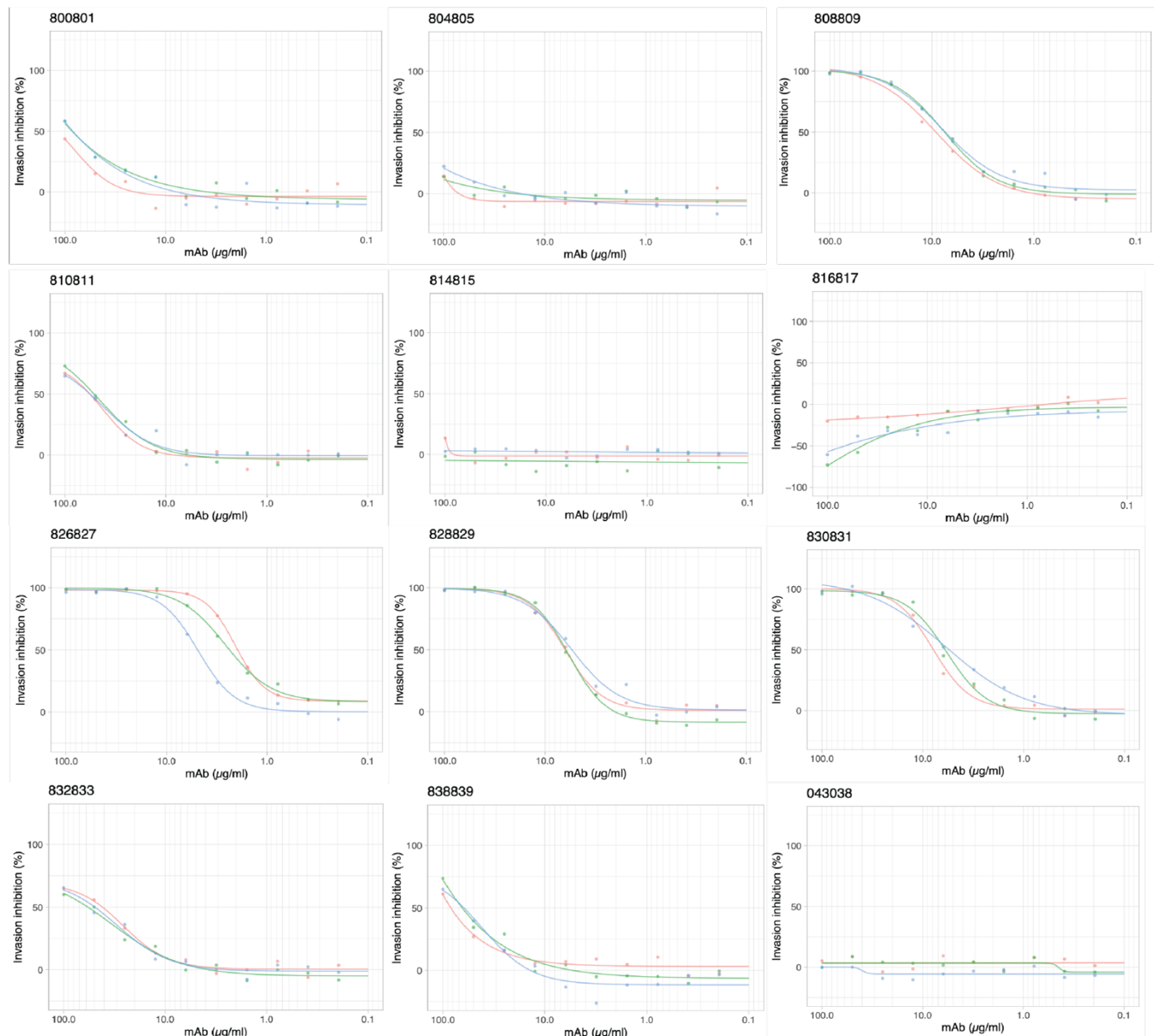


B.

	Kon	Koff
800801	0.00×10^0	0.00×10^0
804805	2.48×10^6	1.14×10^{-2}
806807	1.35×10^6	7.40×10^{-3}
808809	6.01×10^5	-2.35×10^{-3}
810811	8.64×10^5	4.44×10^{-3}
814815	2.56×10^6	5.30×10^{-3}
816817	1.78×10^6	1.46×10^{-2}
826827	6.01×10^5	1.02×10^{-2}
828829	1.28×10^6	1.32×10^{-2}
830831	1.01×10^6	-2.60×10^{-4}
832833	4.77×10^5	8.85×10^{-3}
838839	1.15×10^6	1.42×10^{-2}

Supplemental Figure 4 - Replicates of Pf-PvAMA1 cell line inhibition

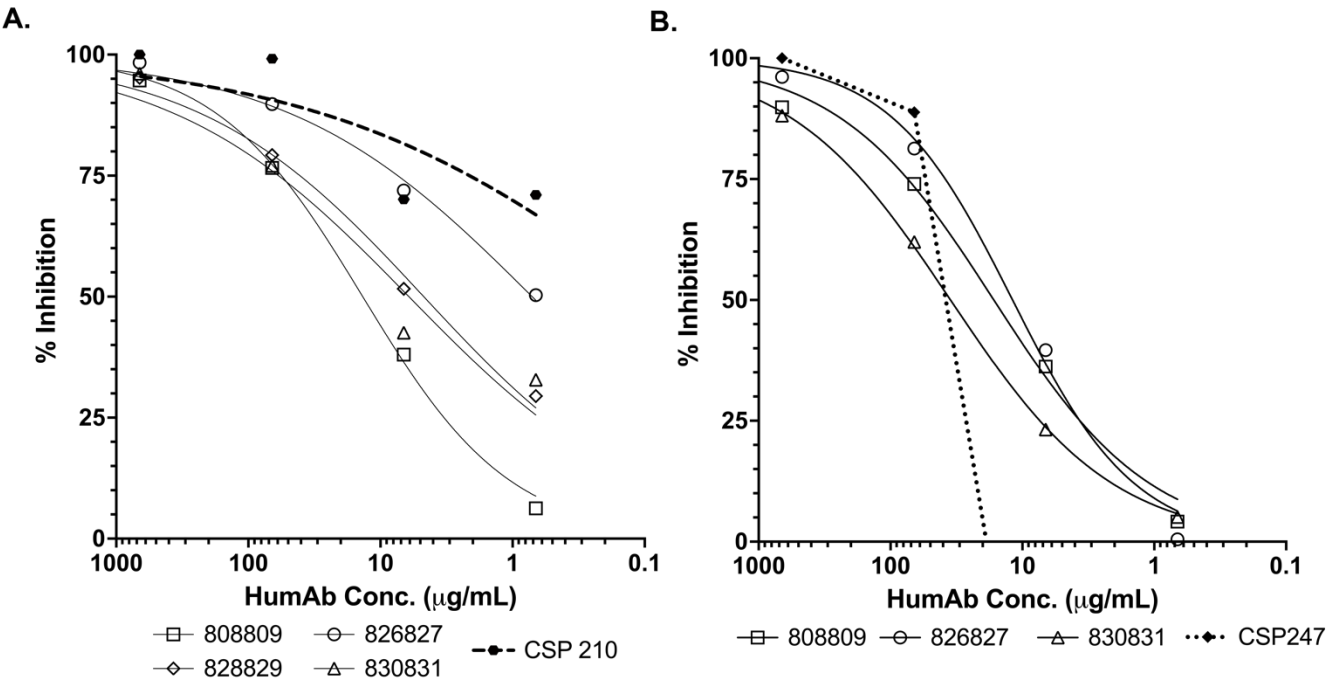
Dose response inhibition curves of PvAMA1-specific humAbs against Pf-PvAMA1 transgenic parasites. Each humAb was tested in triplicate as indicated in different colored lines, and IC₅₀ was calculated using R. 043038, an anti-tetanus toxoid humAb was used as a negative control.



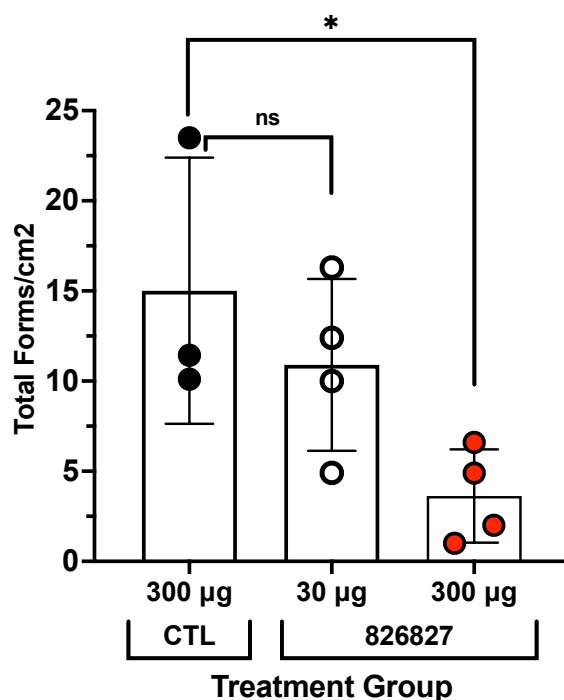
Supplemental Figure 5 – Sporozoite HC04 invasion separated CSP210/247

Dose response inhibition curves of Pv sporozoites blocking by PvAMA1 specific humAbs. Murine anti-CSP monoclonal antibodies served as positive control of blocking inhibition. Three different sporozoite isolates were used for this assay. Based on the blocking activity with the anti-CSP monoclonal one can separate two CSP210 and one CSP247 experiments. Shown below in **A**) are the dose-responses obtained with CSP210 strains and in **B**) with strain CSP247. The calculated IC₅₀ for CSP210 is 0.08 μg/mL and for CSP247 IC₅₀ ~8 μg/mL. HumAbs against PvAMA1 were randomly screened with these isolates. Of note, humAb8 26827

was screened with both sporozoite strains and shows potent inhibition in both in contrast to the CSP monoclonal.

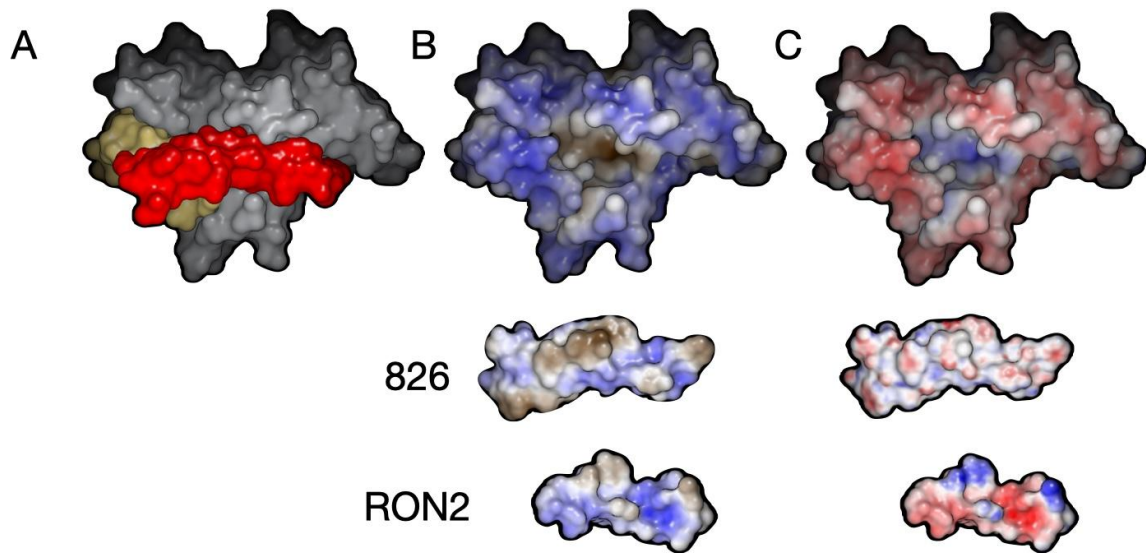


Supplemental Figure 6 – Microscopic assessment of *P. vivax* liver in FRGN huHep mice after administration of anti-AMA1 human monoclonal antibody 826827. The experimental design was identical to that described in the legend of Figure 4. On day 9, liver sections were analyzed microscopically for the presence of parasites described by (Mikolajczak et al. CHM, 2015). Both hypnozoites and schizonts were observed. This analysis shows total parasite forms in the liver (hypnozoites plus schizonts). Of note, one of the control livers could not be adequately evaluated and was not included in the analysis. Each dot represents one mouse. Shown in mean $\pm$ SD. Statistics: unpaired t-test.



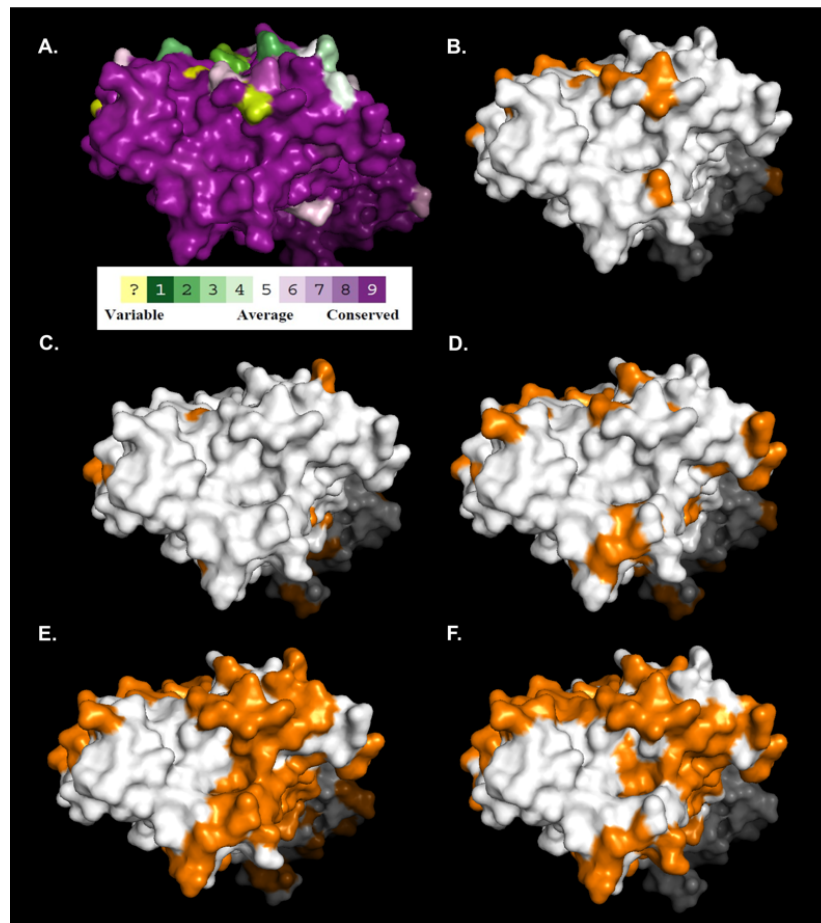
Supplemental Figure 7 - Surface properties of PvAMA1 and its interaction partners RON2 and humAb 826827

A) Schematic overview indicating the Domain 1 of PvAMA1 in gray with the Domain 2 loop in golden and the bound CDR3 loop of 826 in red. **B)** Hydrophobic surface potential of the PvAMA1 binding site. 826 was removed and rotated by 180° compared to the orientation in A to show the corresponding bottom interface of the interaction as well as the corresponding PvRON2 peptide. Darker areas represent higher hydrophobicity. Blue areas show hydrophilicity. **C)** Surface potential of the binding site. Red indicates negatively charged areas. Blue indicates positively charged areas. Figures were generated with Vida 4.4 from OpenEye.



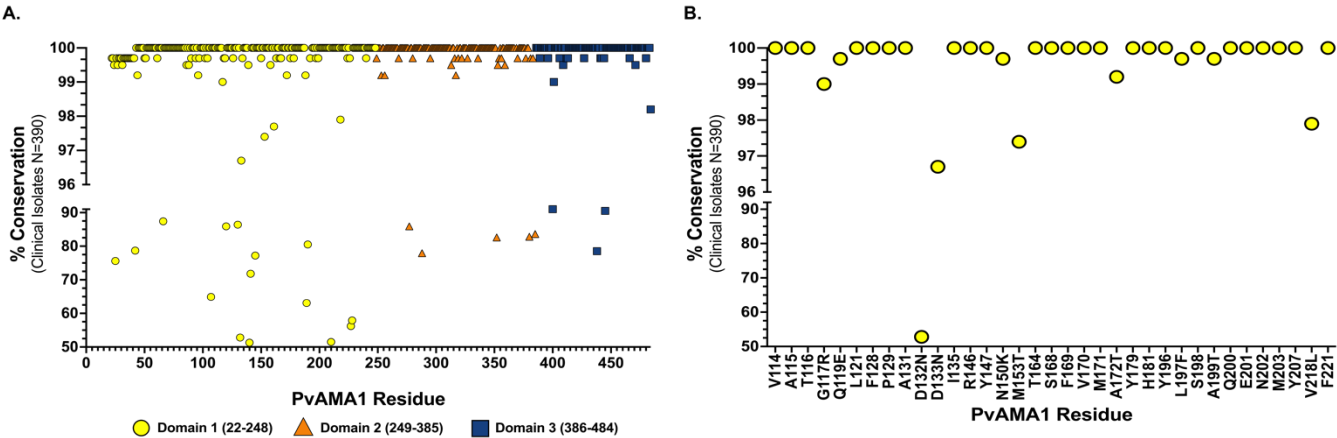
Supplemental Figure 8 – RON2 binding groove conservation and Sequence differences between Pv, PvPNG16, Pf, Pc, Pk, Tg

For all images, a residue-colored orange indicates an amino acid change between PvAMA1_PaloAlto (PDB: 9DX6) and another species' version of AMA1 **A)** Sequence conservation of residues surrounding the RON2 binding groove across 390 published clinical isolates **B)** PvAMA1_PaloAlto versus PvAMA1_PNG16 **C)** PvAMA1_PaloAlto versus *P. cynomolgi* **D)** PvAMA1_PaloAlto versus *P. knowlesi* **E)** PvAMA1_PaloAlto versus *P. falciparum* **F)** PvAMA1_PaloAlto versus *Toxoplasma gondii*.



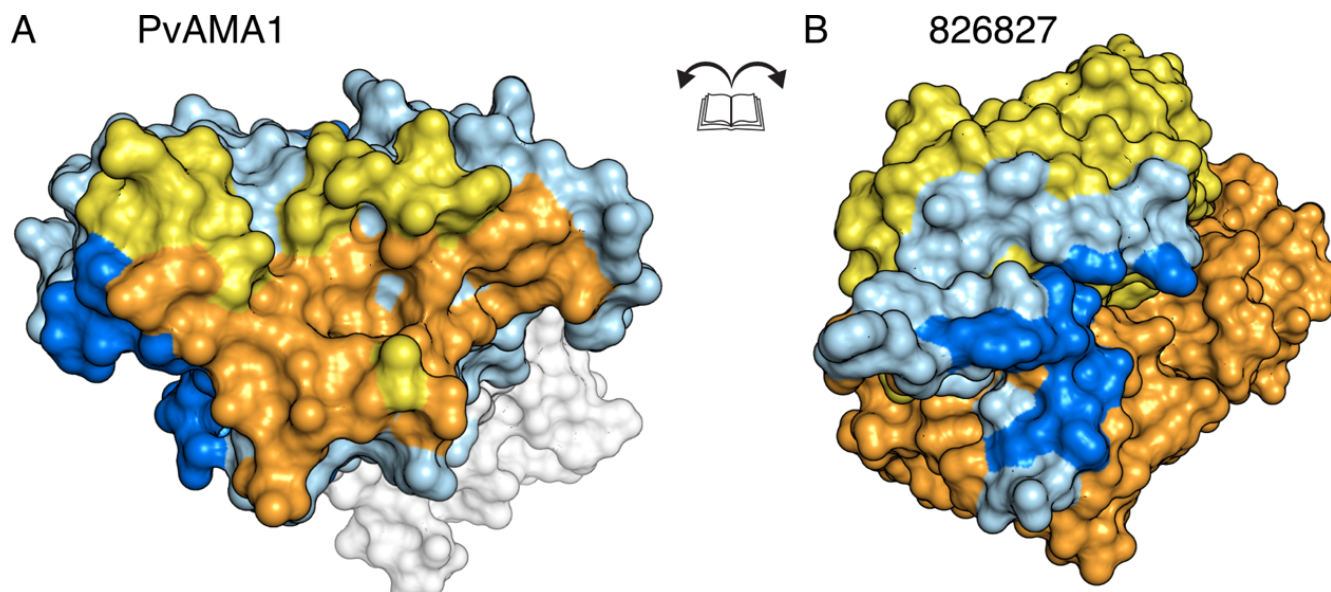
Supplemental Figure 9 – Published PvAMA1 clinical isolates sequence conservation

A) Percent conservation of each amino acid of PvAMA1 across 390 published clinical isolates. Yellow circles represent Domain 1 residues. Orange triangles represent Domain 2 residues. Blue squares represent Domain 3 residues. **B)** Percent conservation of PvAMA1 residues that contact the RON2 extracellular loop.

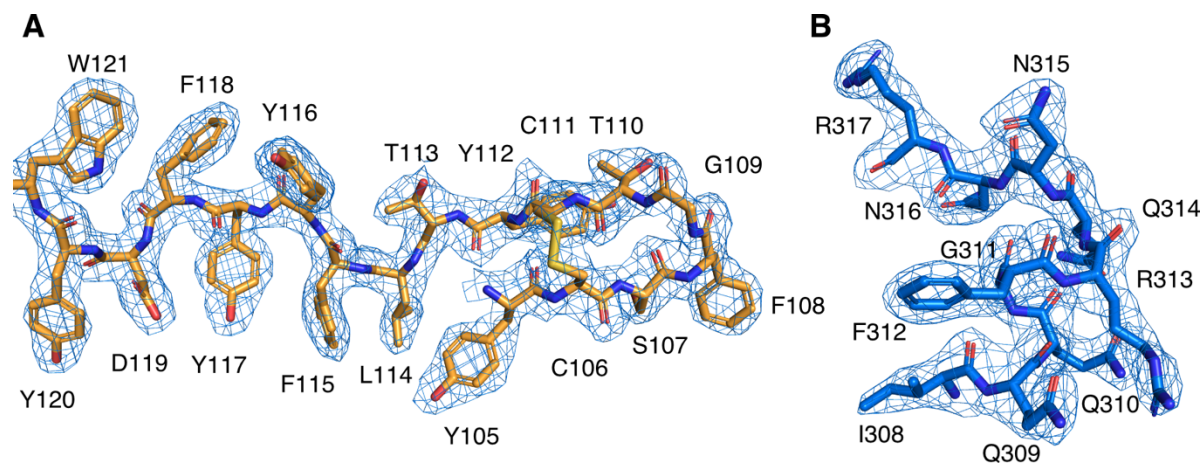


Supplementary Figure 10. Epitope and paratope of the PvAMA1-826827 complex.

(A) Surface representation of PvAMA1 with the epitope recognized by 826827 colored in yellow (LC contribution) and orange (HC contribution). (B) Surface representation of humAb 826827 with the paratope of PvAMA1 colored light blue (domain 1 contribution) and blue (domain 2 loop contribution). Residues within a distance cut-off of 5 Å are highlighted.



Supplementary Figure 11. Representative 2Fo-Fc electron density map of the PvAMA1-826827 crystal structure. The final 2Fo-Fc electron density map (blue, contoured at 1.0 σ) of CDR-H3 residues 105-121 (A), and PvAMA1 residues 308-317 of the D2-loop (B).



Supplemental Table 1 – VDJ alleles and CDR3 sequences and clonal groups

Shown are VDJ alleles and CDR3 sequences for 157 sorted and sequenced Bc IgH. The first column shows B cells that have been made into monoclonal antibodies, assigned a number, and the number of B cells in the clonal group.

# in Clonal Group	HumAb (if produced)	V-GENE & allele	D-GENE & allele	J-GENE & allele	CDR3	# in Clonal Group	HumAb (if produced)	V-GENE & allele	D-GENE & allele	J-GENE & allele	CDR3
1		IGHV4-30-4	IGHD3-10	IGHJ4	CARGSYIRPFDDYW			IGHV5-10-1	IGHD3-10	IGHJ6	CARLSMVQGGGIDVW
		IGHV4-31	IGHD1-26	IGHJ4	CARRSGNYYDYFDYW	3		IGHV5-10-1	IGHD3-10	IGHJ6	CARLSMVQGGGIDVW
3		IGHV4-31	IGHD1-26	IGHJ4	CARRSGNYYDYFDYW			IGHV5-10-1	IGHD3-10	IGHJ6	CARLSMVQGGGIDVW
		IGHV4-31	IGHD1-26	IGHJ4	CARRSGNYYDYFDYW			IGHV5-10-1	IGHD3-10	IGHJ3	CARITMTWGGAFEIW
2		IGHV4-31	IGHD4-11	IGHJ5	CAREDNRNYGRSCFDYW	4		IGHV5-10-1	IGHD2-8	IGHJ3	CARITLTWGGTFDFW
	806807	IGHV4-31	IGHD4-11	IGHJ5	CAREDNRNYGRSCFDYW		838839	IGHV5-10-1	IGHD2-8	IGHJ3	CARITLTWGGTFDFW
1		IGHV4-39	IGHD5-12	IGHJ5	CARQQVSAYHSRPGWDFPW			IGHV5-10-1	IGHD1-26	IGHJ3	CARSLTWGGTLDIW
1		IGHV4-39	IGHD5-24	IGHJ4	CARQGRGGYDWRPRGPDYW	1	804805	IGHV3-30-5	IGHD2-2	IGHJ4	CAKELYAYSTSPLDNW
		IGHV4-31	IGHD3-22	IGHJ5	CARASSYYDSSGYYYNWDFPW	1		IGHV3-33	IGHD6-13	IGHJ5	CARGRGGOHESWDFPW
2		IGHV4-31	IGHD3-22	IGHJ5	CARASSYYDSSGYYYNWDFPW	1		IGHV3-30	IGHD4-11	IGHJ6	CARDRLSATTYGYFEYW
		IGHV4-61	IGHD2-15	IGHJ4	CARAHGEGHCSGGSCYSLFYFDYW			IGHV3-30	IGHD3-9	IGHJ4	CAKSRPAYTLTAPFDSW
4		IGHV4-61	IGHD2-15	IGHJ4	CARARGEAYCSGGTCYTLFYFDYW	4		IGHV3-30	IGHD3-9	IGHJ4	CAKSRPAYTLTAPFDSW
		IGHV4-61	IGHD2-15	IGHJ4	CARAHGEGYCSGGSCYSLFYFDYW			IGHV3-30	IGHD3-9	IGHJ4	CAKNRPTTLTAPFDSW
		IGHV4-61	IGHD2-15	IGHJ4	CARAYGEGYCSFGTCYTLFYFDNW			IGHV3-30	IGHD3-9	IGHJ4	CAKNRPTTLTAPFDSW
		IGHV4-61	IGHD2-15	IGHJ4	CARGHGEYCSGGTCYSLFYFEYW	1		IGHV3-30	IGHD3-3	IGHJ4	CAKGRGSFTLTTPFDYW
		IGHV4-61	IGHD2-15	IGHJ4	CARGHGEYCSGGTCYSLFYFEYW	1		IGHV3-30	IGHD4-17	IGHJ4	CAKDSGVATMGGAHYHYW
		IGHV4-61	IGHD2-15	IGHJ4	CARGRGEYCSGGNCTYLFYFDYW	1		IGHV5-10-1	IGHD5-18	IGHJ2	CARRGTSMGPDDWYFDLW
7		IGHV4-61	IGHD2-15	IGHJ4	CARSRGEGYCSFGTCYTLFYFDYW			IGHV5-10-1	IGHD3-3	IGHJ4	CARLSVTFLSGFPDYW
	826827	IGHV4-61	IGHD2-15	IGHJ4	CARSRGEGYCSFGTCYTLFYFDYW			IGHV5-10-1	IGHD3-3	IGHJ4	CARLSVTFLSGFPDYW
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		IGHV4-61	IGHD2-15	IGHJ4	CVSANGEGYCSGGTCYSLFYFDYW			IGHV5-10-1	IGHD3-3	IGHJ4	CARLSVTFLSGFPDHW
1	830831	IGHV4-30-4	IGHD2-21	IGHJ6	CARSVGDGYCSGGACFFLYTGLDWW			IGHV5-10-1	IGHD3-3	IGHJ4	CARLSVTFLSGFPDHW
		IGHV3-15	IGHD2-21	IGHJ6	CTTNVRSATGYGGLAVW			IGHV5-10-1	IGHD3-22	IGHJ4	CARLEGLYDSSGYFGYW
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		IGHV3-11	IGHD4-11	IGHJ5	CARALEYANTWYPYW			IGHV3-48	IGHD3-22	IGHJ6	CARLAAYYDTGAYRTDYFYAMDVW
		IGHV3-11	IGHD4-11	IGHJ5	CARALEYANTWYPYW	1		IGHV3-48	IGHD3-22	IGHJ6	CARVMMHYDASAYRTDYFYAMDVW
		IGHV3-11	IGHD4-11	IGHJ5	CARALEYANTWYPYW	2		IGHV3-69	IGHD3-22	IGHJ6	CARLAAYYDTGAYRTDYFYAMDVW
		IGHV3-11	IGHD4-11	IGHJ5	CARALEYANTWYPYW			IGHV3-69	IGHD3-22	IGHJ6	CARLAAYYDTGAYRTDYFYAMDVW
		IGHV3-11	IGHD4-11	IGHJ5	CARALDYANTLYPLW			IGHV5-10-1	IGHD2-15	IGHJ6	CARLYSGANCYSGGTYYYHVDVW
2		IGHV3-74	IGHD5-23	IGHJ4	CARAGHYVSPNFDYW	4		IGHV5-10-1	IGHD2-15	IGHJ6	CARLYSGANCYSGGTYYYHVDVW
		IGHV3-74	IGHD5-24	IGHJ4	CARAGHYVSPNFDYW			IGHV5-10-1	IGHD2-15	IGHJ6	CARLYSGANCYSGGTYYYHVDVW
		IGHV5-10-1	IGHD3-10	IGHJ4	CARVSLVRGVLDYW			IGHV5-10-1	IGHD2-15	IGHJ6	CARLYSGANCYSGGTYYYHVDVW
		IGHV5-10-1	IGHD3-10	IGHJ4	CARMTMVRGVLDYW	1		IGHV1-46	IGHD2-15	IGHJ6	CARDRSAGGSWTGSLYHYGLGW
1		IGHV5-10-1	IGHD3-3	IGHJ3	CARLTLLGGNAFDIW	1	828829	IGHV4-39	IGHD3-22	IGHJ5	CARRSAEGYCSGGSCYSLWGVGFGPW
1	832833	IGHV1-3	IGHD2-21	IGHJ3	CAREYCDTGRCLAGVVVPAEAFDIW						

Supplemental Table 2 – Avidity Index (AI₅₀) of PvAMA1 specific humAbs to different AMA1 constructs

Al₅₀	800801	804805	806807	808809	810811	814815	816817	826827	828829	830831	832833	838839
PvAMA1_Palo Alto	1.01	1.93	3.30	0.80	0.88	1.26	1.40	2.25	1.12	1.12	2.77	1.64
PvAMA1_PNG16	0.96	2.57	2.93	1.04	0.87	0.76	0.93	2.08	0.78	0.53	3.00	1.43
PkAMA1	0.95	0.46	3.10	1.23	0.03	0.00	1.37	3.87	0.89	2.16	0.78	2.21

Supplemental Table 3 - List of SNP and haplotypes observed in AMA1 sequences in isolates tested in response to 826827

[illegible]

Supplemental Table 4 – Crystallography data collection and refinement statistics

	PvAMA1-Fab 826827
PDB ID	9DX6
Data collection statistics	
Wavelength (Å)	0.953725
Space group	C121
Cell axes (Å) (a, b, c)	187.24, 54.46, 104.13
Cell angles (°) (α, γ, β)	90, 97.20, 90
Resolution range (Å)	47.76-2.40 (2.54-2.40)*
Completeness (%)	99.9 (99.8)
Total no. of reflections	289692 (45358)
Unique reflections	41378 (6614)
Redundancy	7.0 (6.9)
R _{meas} (%)	18.0 (117.6)
CC _{1/2} (%)	99.5 (64.2)
I/σ	9.53 (1.55)
Wilson B (Å ²)	42.79
Refinement statistics	
R _{work} /R _{free} (%)	19.2/ 23.5
<u>No. of atoms</u>	
Protein	6395
Water	259
<u>B factors (Å²)</u>	
Chain A	46.8
Chain B	45.3
Chain C	49.6
Water	45.7
<u>R.m.s. deviations</u>	
Bond lengths (Å)	0.004
Bond angles (°)	0.668
Validation	
<u>Ramachandran plot</u>	
outliers (%)	0.0
favoured (%)	96.6
Rotamer outliers (%)	0.9
C-beta outliers	0
MolProbity score	1.49

* The values in parentheses represent the highest-resolution shell.

Supplemental Table 5 - Interactions PvAMA1 – Fab 826827 based on PISA

PvAMA1	Group	826827	Location	Group	Distance
Hydrogen bonds					
Glu 83	OE2	Tyr 54	CDR-H2	OH	2.4
Glu 83	OE2	Tyr 55	CDR-H2	OH	3.3
Thr 116	OG1	Tyr 49	CDR-L2	OH	2.9
Asp 118	OD1	Gly 57	CDR-L2	N	3.0
Asp 118	OD2	Arg 54	CDR-L2	NH2	2.8
Asp 118	N	Arg 54	CDR-L2	O	2.9
Asp 118	O	Arg 54	CDR-L2	NH2	3.7
Ala 131	O	Lys 31	CDR-L1	N	2.9
Ala 131	O	Thr 32	CDR-L1	N	3.5
Asn 132	ND2	Thr 113	CDR-H3	O/N	2.8/ 3.5
Asp 133	N	Ser 30	CDR-L1	OG	2.9
Val 170	O	Cys 106	CDR-H3	N	2.7
Val 170	N	Cys 106	CDR-H3	O	2.7
Ala 172	N	Gly 104	CDR-H3	O	2.9
Tyr 196	OH	Arg 101	CDR-H3	NH2	3.5
Tyr 196	OH	Glu 103	CDR-H3	OE1/OE2	2.8/2.6
Gln 314	O	Tyr 34	CDR-H1	OH	3.6
Asn 315	ND2	Tyr 117	CDR-H3	OH	2.8
Asn 315	O	Arg 101	CDR-H3	NE	2.7
Asn 315	O	Tyr34	CDR-H1	OH	3.8
Asn 315	OD1	Arg 101	CDR-H3	NH2	3.6
Asn 316	ND2	Tyr 34	CDR-H1	OH	3.7
Lys 321	NZ	Tyr 105	CDR-H3	OH	2.6
Salt bridges					
Lys 321	NZ	Glu 103	CDR-H3	OE2	3.6
Asp 118	OD2	Arg 54	CDR-L2	NH2	2.8
Other interfacing residues in PvAMA1					
Asn 84	Gly 117	Gln 119	Phe 128	Pro 129	Asn 130
His 134	Ile 135	Arg 146	Tyr 147	Asn 150	Met 153
Thr 164	His 165	Ser 168	Phe 169	Met 171	Gly 173
Gln 175	His 181	Met 194	Gln 310	Arg 313	Arg 317
Glu 318					
Other interfacing residues in 826 (heavy chain)			Other interfacing residues in 827 (light chain)		
Ser 331	Pro 32	Gly 33	Ser 28	Ser 50	Thr 53
Tyr 35	Arg 56	Arg 99	Ala 55	Ser 56	Val 58
Gly 102	Ser 107	Phe 108	Tyr 91	Asn 92	Trp 94
Cys 111	Tyr 112	Leu 114			
Phe 115	Asp 119	Tyr 120			