Supplementary Information

Structural basis for inhibition of *Plasmodium vivax* invasion by a broadly neutralising vaccine-induced human antibody

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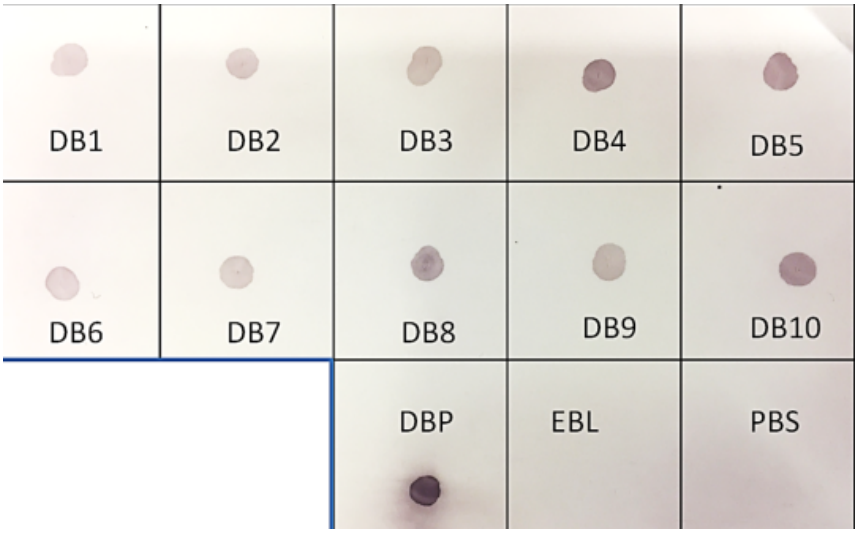
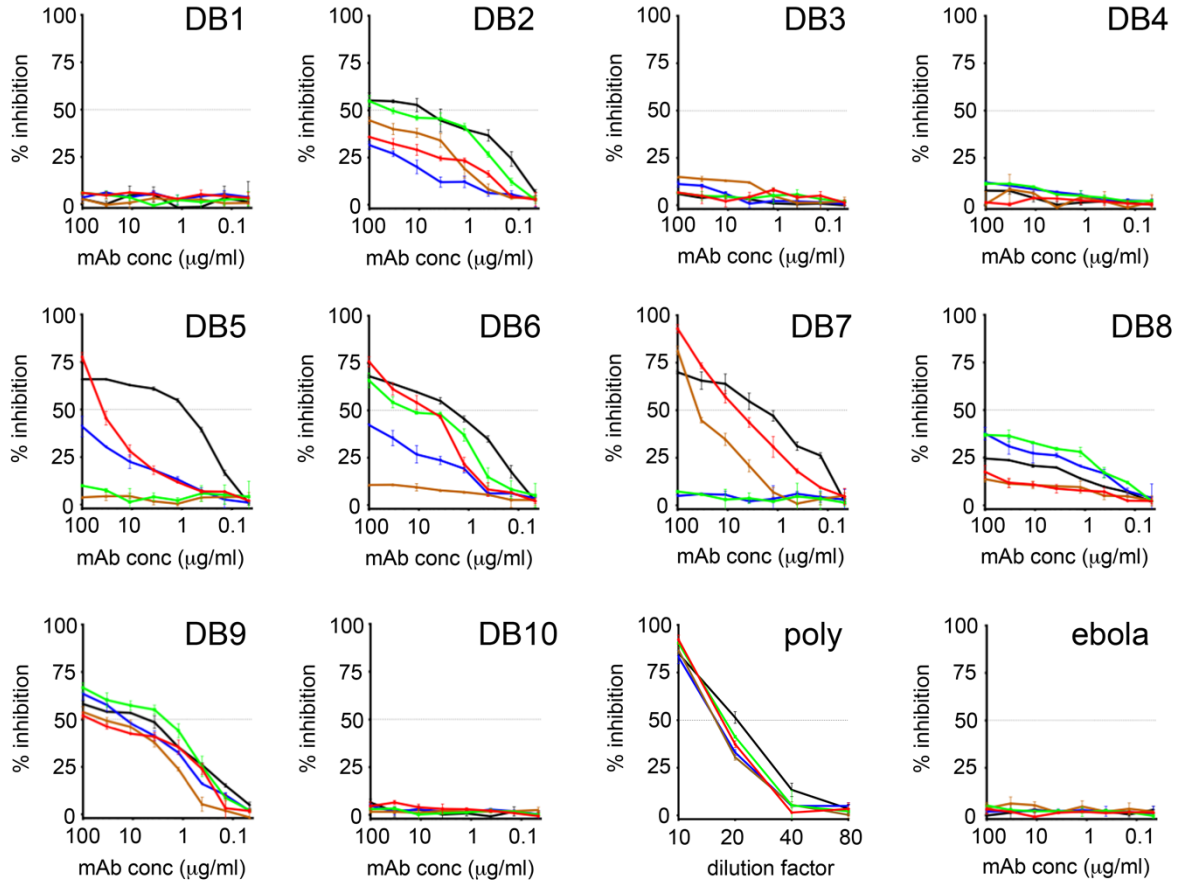
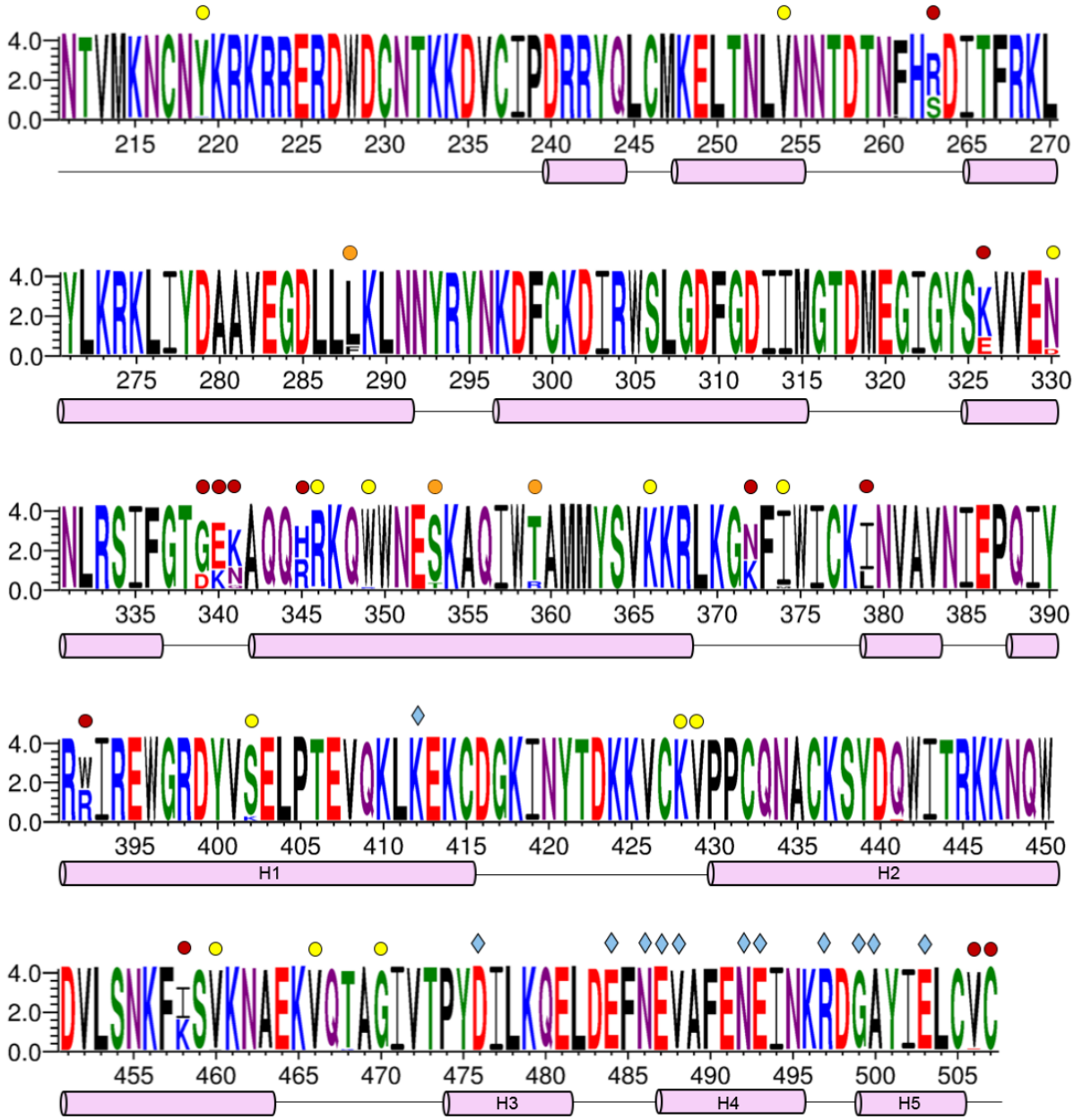


Supplementary Figure 1: Recognition by *Pv*DBP from culture supernatants by dot blot
Dot blot assay showing binding of the ten human anti-*Pv*DBPII mAbs to native *Pv*DBP secreted in the supernatant of a short-term *in vitro* culture of *Plasmodium vivax*. The positive control was recombinant *Pv*DBPII (DBP) and the two negative controls were a human anti- *Ebolavirus* GP IgG1 mAb (EBL) and PBS. This assay was conducted once due to limited quantities of culture supernatant.

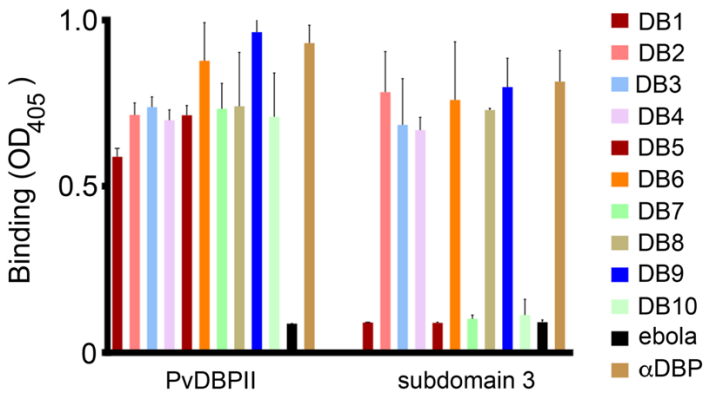


Supplementary Figure 2: Anti-*Pv*DBPII mAb inhibition of the binding of recombinant *Pv*DBPII variants to the recombinant N-terminal 60 amino acid DARC ectodomain

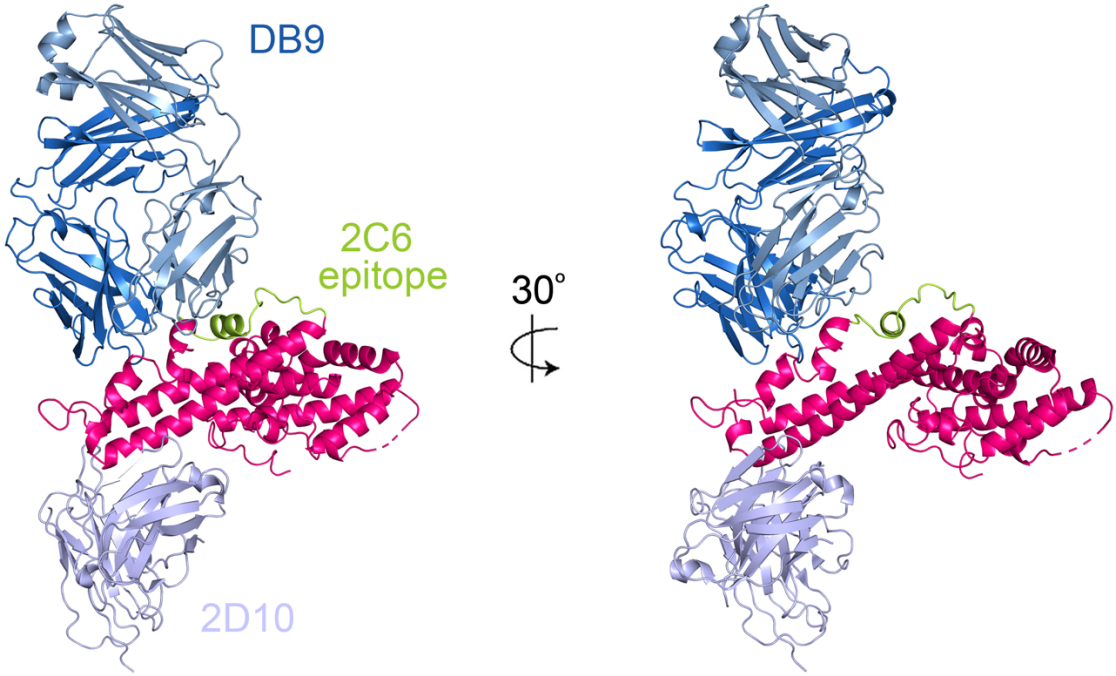
The binding of five naturally occurring variants of *Pv*DBPII to the DARC ectodomain in the presence of increasing concentrations of each of DB1-DB10. The variants are SalI (red), AH (green), O (brown), P (blue) and HMP013 (black). "poly" is polyclonal human anti-*Pv*DBPII serum from the VAC051 clinical trial [19], while "ebola" is an anti-*Ebolavirus* recombinant human IgG1 mAb included as a negative control. Data points represent the mean of three technical replicates, while the error bars represent the standard deviation.



Supplementary Figure 3: Sequence logo representing sequence conservation across *Pv*DBPII
Sequence logo derived from 383 sequences of *Pv*DBPII from *Plasmodium vivax* isolates. Underneath the logo is the residue number from the SalI *Pv*DBPII variant. Cylinders represent the location of helices while lines represent loops. Above the sequence, blue kites indicate residues which directly contact DB9. Yellow, orange and red circles represent residues with sequence entropies of 0.15-0.3, 0.3-0.45 and >0.45 respectively.



Supplementary Figure 4: Analysis of the binding of monoclonal antibodies to *Pv*DBPII subdomain 3
An ELISA of the ten anti-*Pv*DBPII mAbs (at 10 µg/mL) binding to recombinant *Pv*DBPII (left) and subdomain 3 (right). Columns represent the mean of three technical replicates, while the error bars represent the standard deviation and the assay was performed twice with similar results. Human polyclonal anti-*Pv*DBPII serum (αDBP) from the VAC051 clinical trial [19] at 1:100 dilution and a human anti-*Ebolavirus* IgG1 mAb (ebola) at 10 µg/mL were used as positive and negative controls, respectively.



Supplementary Figure 5: Comparison of the epitope for DB9 with those of previously identified mouse monoclonal antibodies
*Pv*DBPII is shown in pink and DB9 in dark blue. The ScFv fragment of mouse antibody 2D10 [26] is shown in lilac while the epitope of 2C6 in *Pv*DBPII, identified by hydrogen-deuterium exchange mass spectrometry is shown in green.

Supplementary Table 1: Genetic lineage of heavy and light chain variable regions from *Pv*DBPII-specific mAbs

The allele usage, amino acid sequences and percentage of nucleotide substitutions relative to germline are shown.

mAb	Chain	Fv allele usage and amino acid sequence	Germline change
DB1	heavy	IGHV5-51*03 IGHD3-22*01 IGHJ3*02 EVQLVQSGAEVKKPGESLKISCKGSGYSFTDYWIGWVRQMPGKG LEWMGIIYAGDSDTRYSPSFQGQVTISADKSISTASLQWSSLKA SDTAMYYCARLAYDSSGYYYAFDIWGQGTMVTVSS	1.7% (5/294)
	light	IGKV1-39*01,IGKV1D-39*01 IGKJ5*01 DIVMTQSPSSLSASVGDRVTITCRASQTISSYLNWYQQKPGKAP KLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC QQSYSTPLITFGQGTRLEIKRTV	1.7% (5/286)
DB2	heavy	IGHV4-59*08 IGHD6-13*01 IGHJ3*02 QVQLQESGPGLVKPSETLSLTCTVSGGSISSYYWSWIRQPPGKG LEWIGYISYTGSTNYNPSLKSRVTISVDTSKNQFSLKLSSVTAA DTAVYSCARHFHSSTAAAFDIWGQGTMVTVSS	1.4% (4/293)
	light	IGKV3-20*01, IGKV3D-20*01, IGKJ1*01 EIVLTQSPGTLSLSPGEGATLSCRASQSVSNSYLAWYQQKPGQA PRLLIYGASIRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYY CQQYGRSPRT	2.1% (6/289)
DB3	heavy	IGHV4-31*03 IGHD2-2*01,IGHD2-2*02,IGHD2-2*03 IGHJ4*02 QLQLVESGPGLVKPSQTLSLTCTVSGGSTSSGGYYWNWIRQHPG KGLEWIGYIHNSGSTYYNPSLKSRGIISVDTSKHQFSLRLRSVT AADTAEYYCARSQGYCSSSSCLLPRGYFDYWGPGILVTVSS	6.4% (19/297)
	light	IGLV1-51*01 IGLJ2*01,IGLJ3*01 QSALTQPPSVSAAPGQKVTISCSGSSSNIGNNFVSWYQLFPGTA PKLLIYDNNERPSGIPDRFSGSRSGTSATLGITGLQTGDEADYY CGTWDSSLSAVVFGGGTKLTVLGQP	3.7% (11/295)
DB4	heavy	IGHV1-69*06 IGHD6-6*01 IGHJ4*02 QLVQSGAEVKKPGSSVKVSCKASGDTSSSYAISWVRQAPGQGLE WMGGIIPIFGTANYAQKFQGRFTITAHKSTSTAYMELSSLRSDD TAVYYCARDGGHHGQLVFDYWGQGTLVTVSS	2.7% (8/295)

	light	IGKV1-16*02 IGKJ4*01 DIQLTQSPSSLSASVGDRVTITCRASQVISNYLAWFQQKPGKAP KSLIYAASSLQSGVPSKFSGSGSGTDFTLTISSLQPEDFATYYC QQYNSYPLTFGGGTKVEIRRTV	1.4% (4/284)
DB5	heavy	IGHV1-46*03 IGHD1-26*01,IGHD4-11*01,IGHD4-4*01 IGHJ6*02 QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQG LEWMGIINPSGGSTSYAQKFQGRVTMTRDTSTSTVYMELSSLRS EDTAVYFCARDNSEGAAYYSYYYYYGMDVWGQGTTVTVSS	0.7% (2/296)
	light	IGLV1-44*01 IGLJ3*02 QSALTQPPSASGTPGQRVTISCSGSSSNIGSNTVNWYQQVPGTA PKLLIYSNNQRPSGVPDRFSGSKSGTSASLAISGLQSEDEADYY CAAWDDSLNGPRFGGGTKLTVLGQP	1% (3/296)
DB6	heavy	IGHV1-2*02 IGHD3-3*02,IGHD5-12*01,IGHD6-13*01 IGHJ6*02 QLVQSGAEVKKPGASVKVSCKASGYSFTGYFLHWVRQAPGQGLE WMGWINPNSGGTKYAQKFQGRVTMTRDTSISTAYMELSRLRSDD TAVYYCARGLRYSIAWYSDYGLDVWGQGTTVTVSS	2.4% (7/294)
	light	IGKV3-20*01 IGKJ4*01 EIVLTQSPGTLSLSPGERATLSCRASQSVTSTYLAWYQQKPGQA PRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYY CQQFGSSLTFGGGTKVEIKRT	1.4% (4/286)
DB7	heavy	IGHV4-39*01 IGHD3-10*01,IGHD3-10*02,IGHD5-18*01 IGHJ5*02 QVQLQESGPGLVKPSETLSLTCTVSGGSIXSISYFWGWIRQPPG KGLEWIGSIYYSGSTYYNPSLKSRVTVSVDTSKNQFSLKLSSVT AADTAVYFCARRSLGYFFGPWGQGTLVTVSS	2.7% (8/298)
	light	IGLV3-21*02 IGLJ2*01,IGLJ3*01 SYELTQPPSVSVAPGQTARITCGGNNIGSKRVHWYQQKPGQAPV LVVYDDSDRPSGIPERFSGSNSGNTATLTIXWVEAGDEADYYCQ LWDTSSDHPVFGGGTKLTVLGX	2.4% (7/290)
DB8	heavy	IGHV1-3*01 IGHD6-19*01 IGHJ4*02 QVQLVQSGAEVKKPGASVKVSCKASGYTFSSYAMHWVRQAPGQR LEWMGWINAGNGNTKYSQKFQDRVTITRDTSASTAYMELSSLSS EDTAVYYCARSYRSSIGWFWMFDYWGQGTLVTVSS	2% (6/294)
	light	IGKV4-1*01 IGKJ2*01 DIVMTQSPDSLGVSLGERATINCKSSQSVLYSSNNKNYLAWYQQ	3% (9/302)

		KPGQPPKLLIYWASTRESGVPDRFSGSGSGTDFTLTITGLQAED VAVYYCLQYYSIPYTFGQGTKVEIKRTV	
DB9	heavy	IGHV4-39*02 IGHD1-1*01,IGHD1-14*01,IGHD1-20*01 IGHJ2*01 EVQLQESGPGLVKPSETLSLTCTVSGGSVSSSTYYWGWVRQPPG KGLEWIGSIYYSGSTYYNPSLKSRVTISVDTSKNQFSLKLSSVT AADTAVYYCARDGTGALDLWGRGTLVTVSS	2.3% (7/298)
	light	IGKV1-5*03 IGKJ1*01 DIVMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQRPGKAP RLLIYKASSLLSGVPSRFGGSGSGTDFTLTISSLQPDDFATYHC QHYNTYPWTFGQGTKVEIKRTV	4.4% (12/274)
DB10	heavy	IGHV3-21*01 IGHD3-10*01,IGHD3-10*02 IGHJ6*02 QVQLVESGGGLVKPGGSLRLSCAASGFTFSTYSMNWVRQAPGKG LEWVSSITSSSSYMDYADSVKGRFTISRDNAKNSLYLQMTSLRA EDTAVYYCARDSVAGPFYYFYAMDVWGQGTTVTVSS	2.4% (7/295)
	light	IGLV2-14*01 IGLJ2*01,IGLJ3*01 NFVLTQPASVSGSPGQSITISCTGTSSDVGGYNFVSWYQQHPGK APKLMIYEVSDRPSGVSNRFSGSKSGNTASLTISGLQAEDEADY YCSSYTSSSTVVFGGGTKLTVLGQP	0.7% (2/284)

Supplementary Table 2: Kinetic parameters for binding of antibodies to *Pv*DBPII

mAb	k_{on} ($M^{-1}s^{-1}$)	k_{off} (s^{-1})	K_D (pM)
DB1	1.88E+07	6.46E-03	344
DB2	9.02E+05	6.05E-04	671
DB3	2.98E+06	4.27E-04	143
DB4	1.30E+06	4.39E-04	337
DB5	2.33E+06	4.33E-04	186
DB6	8.65E+06	2.53E-04	29.2
DB7	1.72E+07	1.13E-04	6.55
DB8	1.73E+06	3.92E-04	227
DB9	1.79E+07	3.57E-04	20.0
DB10	1.06E+07	5.05E-04	47.8

Supplementary Table 3: Data collection and refinement statistics

	PvDBPII:DB9
Data collection	
Space group	$P6_222$
Cell dimensions	
a, *b*, *c* (Å)	173.58, 173.58, 169.88
α, β, γ (°)	90.0, 90.0, 120.0
Resolution (Å)	84.94 – 3.04 (3.09 – 3.04)
R_{PIM}	4.0 (46.9)
$I / \sigma I$	12.8 (1.2)
Completeness (%)	100.0 (100.0)
Redundancy	19.6 (20.1)
Refinement	
Resolution (Å)	3.04
No. reflections	30703
R_{work} / R_{free}	20.0 / 23.8
No. atoms	
Protein	5754
Ligand/ion	0
Water	0
B-factors	
Protein	115.3
R.m.s. deviations	
Bond lengths (Å)	0.01
Bond angles (°)	1.24

All structures were determined from one crystal.

Values in parentheses are for highest-resolution shell.

Supplementary Table 4: List of contacts between *Pv*DBPII and DB9

*Pv*DBPII residue	Group	Fab Chain	Residue	Group	Interaction
K412	Side Chain	Heavy Chain	Y74	Side Chain	Hydrogen bond
D476	Side Chain	Light Chain	S75	Side Chain	Hydrogen bond
E484	Side Chain	Heavy Chain	E20	Main Chain	Hydrogen bond
E484	Side Chain	Heavy Chain	G45	Main Chain	Hydrogen bond
N486	Side Chain	Heavy Chain	R118	Side Chain	Hydrogen bond
E487	Side Chain	Light Chain	Y68	Side Chain	Hydrogen bond
E487	Side Chain	Light Chain	S75	Main Chain	Hydrogen bond
V488	Side Chain	Light Chain	Y68	Side Chain	Hydrophobic
V488	Side Chain	Light Chain	L74	Side Chain	Hydrophobic
N492	Side Chain	Heavy Chain	T121	Main Chain	Hydrogen bond
E493	Side Chain	Heavy Chain	T52	Side Chain	Hydrogen bond
R497	Side Chain	Light Chain	W51	Side Chain	Cation-pi
R497	Main Chain	Heavy Chain	T121	Side Chain	Hydrogen bond
R497	Side Chain	Heavy Chain	T121	Main Chain	Hydrogen bond
G499	Main Chain	Heavy Chain	T52	Main Chain	Hydrogen bond
A500	Main Chain	Heavy Chain	S51	Main Chain	Hydrogen bond
E503	Side Chain	Heavy Chain	Y54	Side Chain	Hydrogen bond
E503	Side Chain	Heavy Chain	Y79	Side Chain	Hydrogen bond

Supplementary Table 5: Summary of the properties of the monoclonal antibodies

	k_{on} (M^{-1} s^{-1})	k_{off} (s^{-1})	K_D (pM)	Inhibitory in protein-based binding assay	Inhibitory in transgenic *P. knowlesi* assay	Inhibitory against homologous *P. vivax* isolates	% of *P. vivax* strains where invasion inhibited	Binds to PvDBPII subdomain 3
DB1	1.88×10^{7}	6.46×10^{-3}	344	no	potent	potent	50	no
DB2	9.02×10^{5}	6.05×10^{-4}	671	yes	low	low	0	yes
DB3	2.98×10^{6}	4.27×10^{-4}	143	no	intermediate	intermediate	0	yes
DB4	1.30×10^{6}	4.39×10^{-4}	337	no	low	low	0	yes
DB5	2.33×10^{6}	4.33×10^{-4}	186	yes	intermediate	n/a	66	no
DB6	8.65×10^{6}	2.53×10^{-4}	29.2	yes	intermediate	n/a	33	yes
DB7	1.72×10^{7}	1.13×10^{-4}	6.55	yes	intermediate	intermediate	50	no
DB8	1.73×10^{6}	3.92×10^{-4}	227	no	low	low	0	yes
DB9	1.79×10^{7}	3.57×10^{-4}	20.0	yes	potent	potent	91	yes
DB10	1.06×10^{7}	5.05×10^{-4}	47.8	no	potent	potent	40	no

Supplementary Table 6: Primers.

Primer number	Primer sequence (5’ to 3’)
1	ACAGGTGCCCACTCCCAGGTGCAG
2	AAGGTGTCCAGTGTGARGTGCAG
3	CCCAGATGGGTCCTGTCCCAGGTGCAG
4	CAAGGAGTCTGTTCCGAGGTGCAG
5	GGAAGGTGTGCACGCCGCTGGTC
6	ATGAGGSTCCCYGCTCAGCTGCTGG
7	CTCTTCCTCCTGCTACTCTGGCTCCCAG
8	ATTTCTCTGTTGCTCTGGATCTCTG
9	GTTTCTCGTAGTCTGCTTTGCTCA
10	GGTCCTGGGCCCAGTCTGTGCTG
11	GGTCCTGGGCCCAGTCTGCCCTG
12	GCTCTGTGACCTCCTATGAGCTG
13	GGTCTCTCTCSCAGCYTGTGCTG
14	GTTCTTGGGCCAATTTTATGCTG
15	GGTCCAATTCYCAGGCTGTGGTG
16	GAGTGGATTCTCAGACTGTGGTG
17	CACCAGTGTGGCCTTGTTGGCTTG
18	CTTTTTCTAGTAGCAACTGCAACCGGTGTACATTCCGAGGTGCAGCTGGTGCAG
19	CTTTTTCTAGTAGCAACTGCAACCGGTGTACATTCTGAGGTGCAGCTGGTGGAG
20	CTTTTTCTAGTAGCAACTGCAACCGGTGTACATTCCCAGGTGCAGCTGCAGGAG
21	CTTTTTCTAGTAGCAACTGCAACCGGTGTACATTCTGAGGTGCAGCTGTTGGAG
22	CTTTTTCTAGTAGCAACTGCAACCGGTGTACATTCCCAGGTGCAGCTACAGCAGTG
23	CTTTTTCTAGTAGCAACTGCAACCGGTGTACATTCCCAGGTTCAGCTGGTGCAG
24	CTTTTTCTAGTAGCAACTGCAACCGGTGTACATTCCCAGGTCCAGCTGGTACAG
25	CTTTTTCTAGTAGCAACTGCAACCGGTGTACATTCTGAAGTGCAGCTGGTGGAG
26	CTTTTTCTAGTAGCAACTGCAACCGGTGTACATTCCCAGGTACAGCTGCAGCAG
27	CTTTTTCTAGTAGCAACTGCAACCGGTGTACATTCCCAGCTGCAGCTGCAGGAG
28	CTTTTTCTAGTAGCAACTGCAACCGGTGTACATTCTCAGGTGCAGCTGGTGGAG
29	GATGGGCCCTTGGTCGACGCTGAGGAGACGGTGACCAG
30	GATGGGCCCTTGGTCGACGCTGAAGAGACGGTGACCATTG
31	GATGGGCCCTTGGTCGACGCTGAGGAGACGGTGACCGTG
32	CTTTTTCTAGTAGCAACTGCAACCGGTGTACATTCTGACATCCAGATGACCCAGTC
33	CTTTTTCTAGTAGCAACTGCAACCGGTGTACATTCAGACATCCAGTTGACCCAGTCT
34	CTTTTTCTAGTAGCAACTGCAACCGGTGTACATTGTGCCATCCGGATGACCCAGTC
35	CTTTTTCTAGTAGCAACTGCAACCGGTGTACATGGGGATATTGTGATGACCCAGAC
36	CTTTTTCTAGTAGCAACTGCAACCGGTGTACATGGGGATATTGTGATGACTCAGTC
37	CTTTTTCTAGTAGCAACTGCAACCGGTGTACATTCAGAAATTGTGTTGACACAGTC
38	CTTTTTCTAGTAGCAACTGCAACCGGTGTACATTCAGAAATAGTGATGACGCAGTC

39	CTTTTTCTAGTAGCAACTGCAACCGGTGTACATTCAGAAATTGTGTTGACGCAGTCT
40	CTTTTTCTAGTAGCAACTGCAACCGGTGTACATTCGGACATCGTGATGACCCAGTC
41	ATGGTGCAGCCACCGTACGTTTGATYTCCACCTTGGTC
42	ATGGTGCAGCCACCGTACGTTTGATATCCACTTTGGTC
43	ATGGTGCAGCCACCGTACGTTTAATCTCCAGTCGTGTC
44	ATGGTGCAGCCACCGTACGTCTGATTTCCACCTTGGTC
45	CTTTTTCTAGTAGCAACTGCAACCGGTTCCTGGGCCCAGTCTGTGCTGACKCAG
46	CTTTTTCTAGTAGCAACTGCAACCGGTTCCTGGGCCCAGTCTGCCCTGACTCAG
47	CTTTTTCTAGTAGCAACTGCAACCGGTTCTGTGACCTCCTATGAGCTGACWCAG
48	CTTTTTCTAGTAGCAACTGCAACCGGTTCTCTCTCSCAGCYTGTGCTGACTCA
49	CTTTTTCTAGTAGCAACTGCAACCGGTTCTTGGGCCAATTTTATGCTGACTCAG
50	CTTTTTCTAGTAGCAACTGCAACCGGTTCCAATTCYCAGRCTGTGGTGACYCAG
51	GGCTTGAAGCTCCTCACTCGAGGGYGGGAACAGAGTG