

Supplementary data for

Comprehensive analysis of Fc-mediated IgM binding to the *Plasmodium falciparum* erythrocyte membrane protein 1 family in three parasite clones

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Figure S1. Erasure of epigenetic memory by pVBH transfection

Relative proportions of *var* gene transcripts in *P. falciparum* 3D7 (top), HB3 (center), and IT4 (bottom) before pVBH transfection (left), after transfection under blasticidin drug pressure (center), and two weeks after release from drug pressure (right). * As detailed in ref. 1.

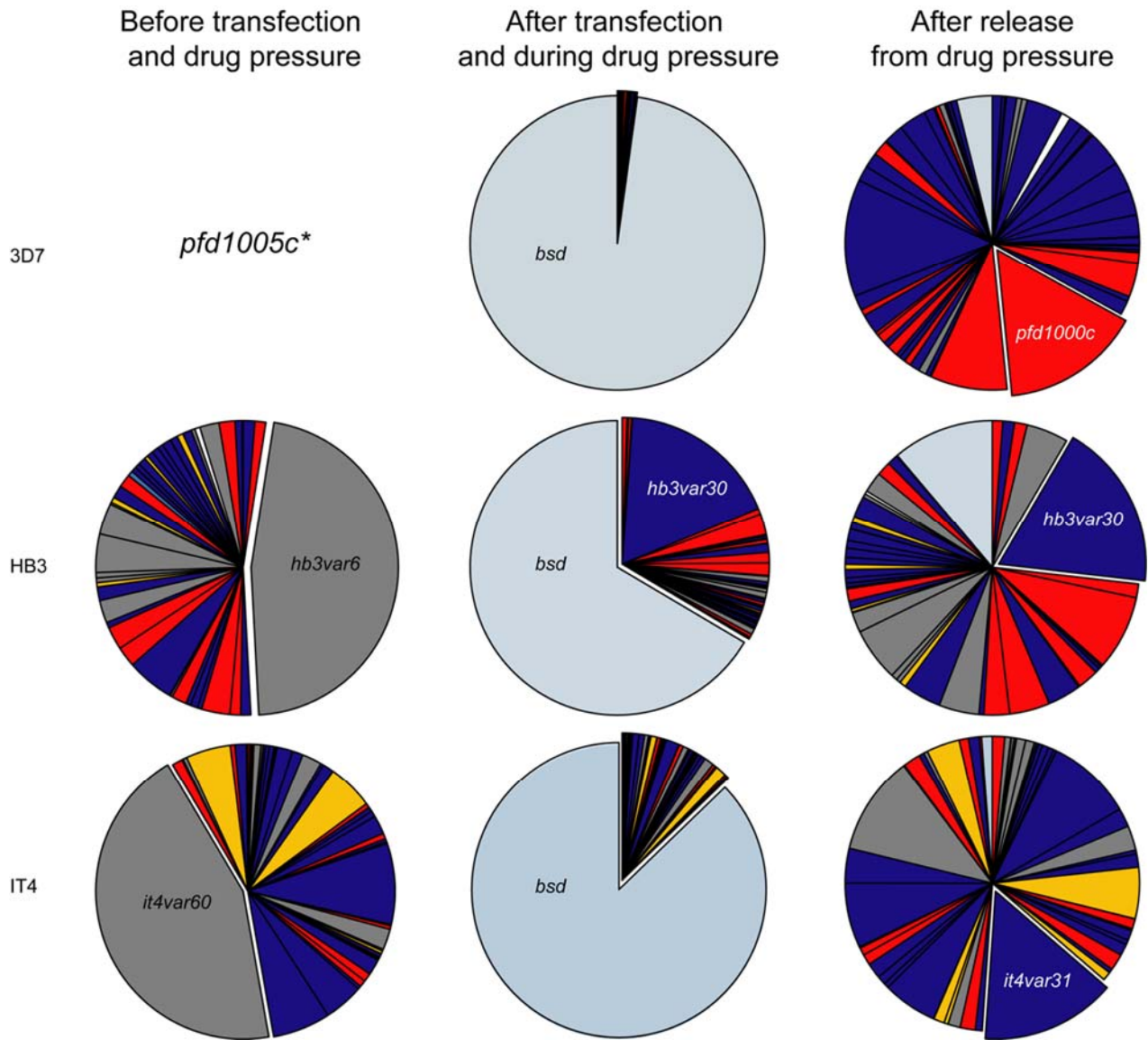


Figure S2. Transcription of var genes in selected *P. falciparum* 3D7, HB3 and IT4 sub-clones

Data presented as in Fig. 2.

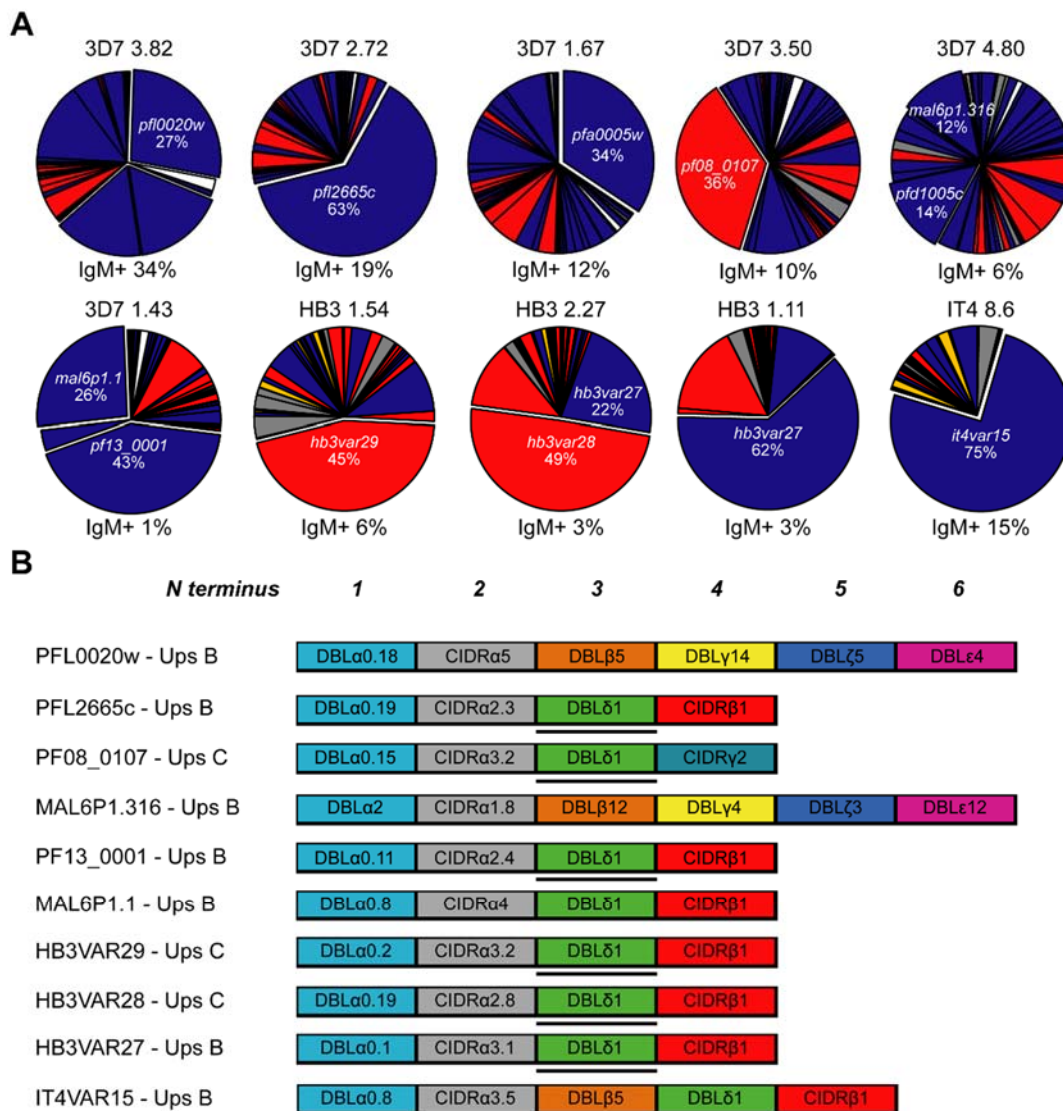


Figure S3. PfEMP1 domains binding to non-immune IgM on ELISA

Data presented as in Fig. 3.

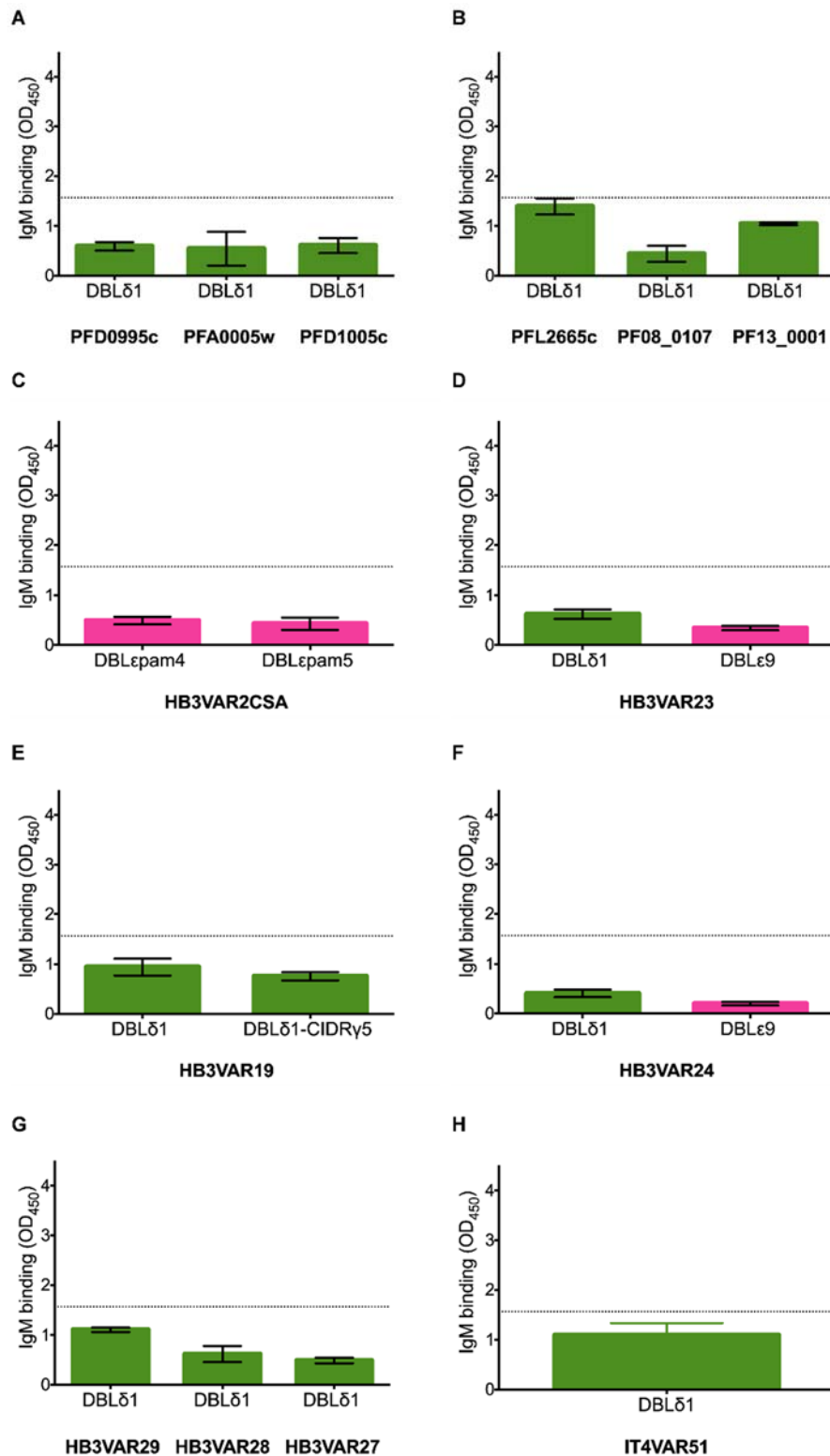


Figure S4. PfEMP1 domains binding to non-immune IgM in a “reverse” ELISA (IgM-coated plates)

Binding of recombinant PfEMP1 domains to immobilized non-immune IgM. Means and standard deviations of data from three independent experiments are shown. Dotted lines represent the cut-off above which binding to non-immune IgM was considered as positive.

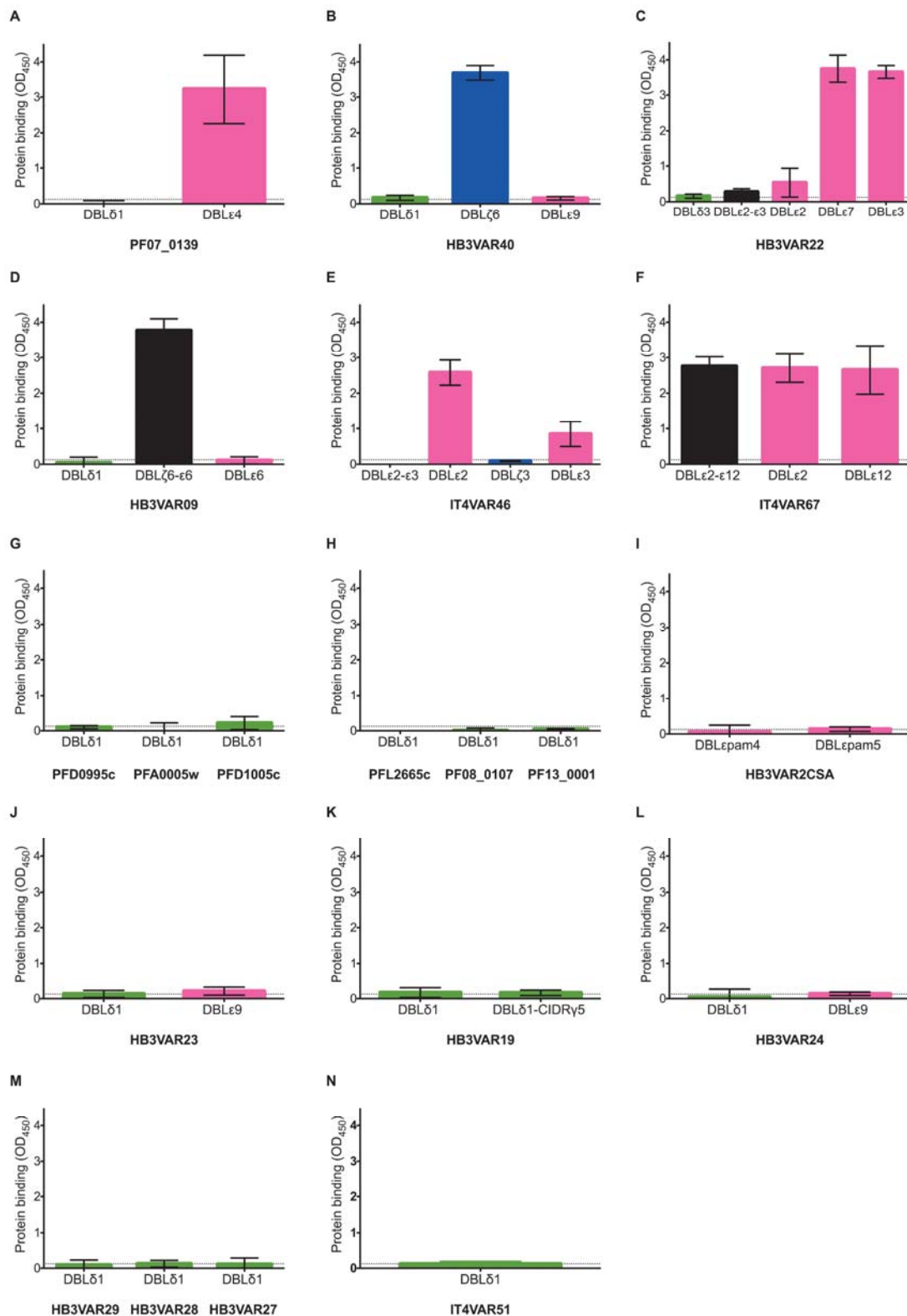
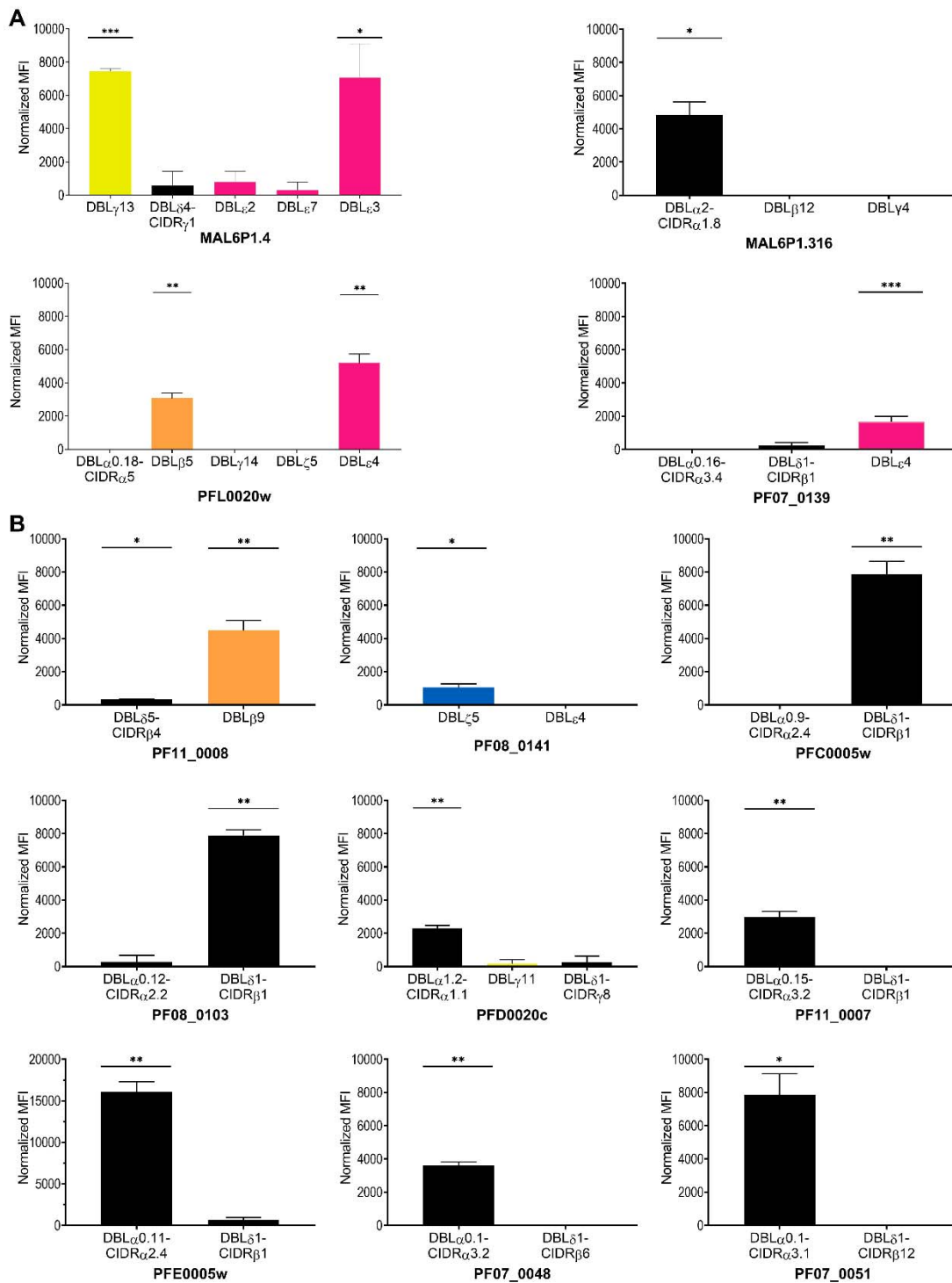


Figure S5. Non-immune IgM-binding to recombinant PfEMP1 domains on BioPlex beads

Non-immune IgM-binding to recombinant domains from PfEMP1 proteins previously (A) or not previously (B) shown to bind IgM. Data shown are the mean fluorescence intensity (MFI) for each recombinant PfEMP1 construct minus the MFI of the control construct measured in the same well. P-value calculated by t-test relative to control construct. Each experiment was repeated at least three times with quantitatively similar results. Mean and standard deviations of representative experiments are shown. Color-coding as in Fig. 2B. * P < 0.05, ** P < 0.01, *** P < 0.001. The domain structures of the PfEMP1 variants involved are also shown (C).



C



Figure S6. Non-immune IgM-binding and specific PfEMP1 expression on the surface of erythrocytes infected by *P. falciparum* sub-clones

Data presented as in Fig. 5.

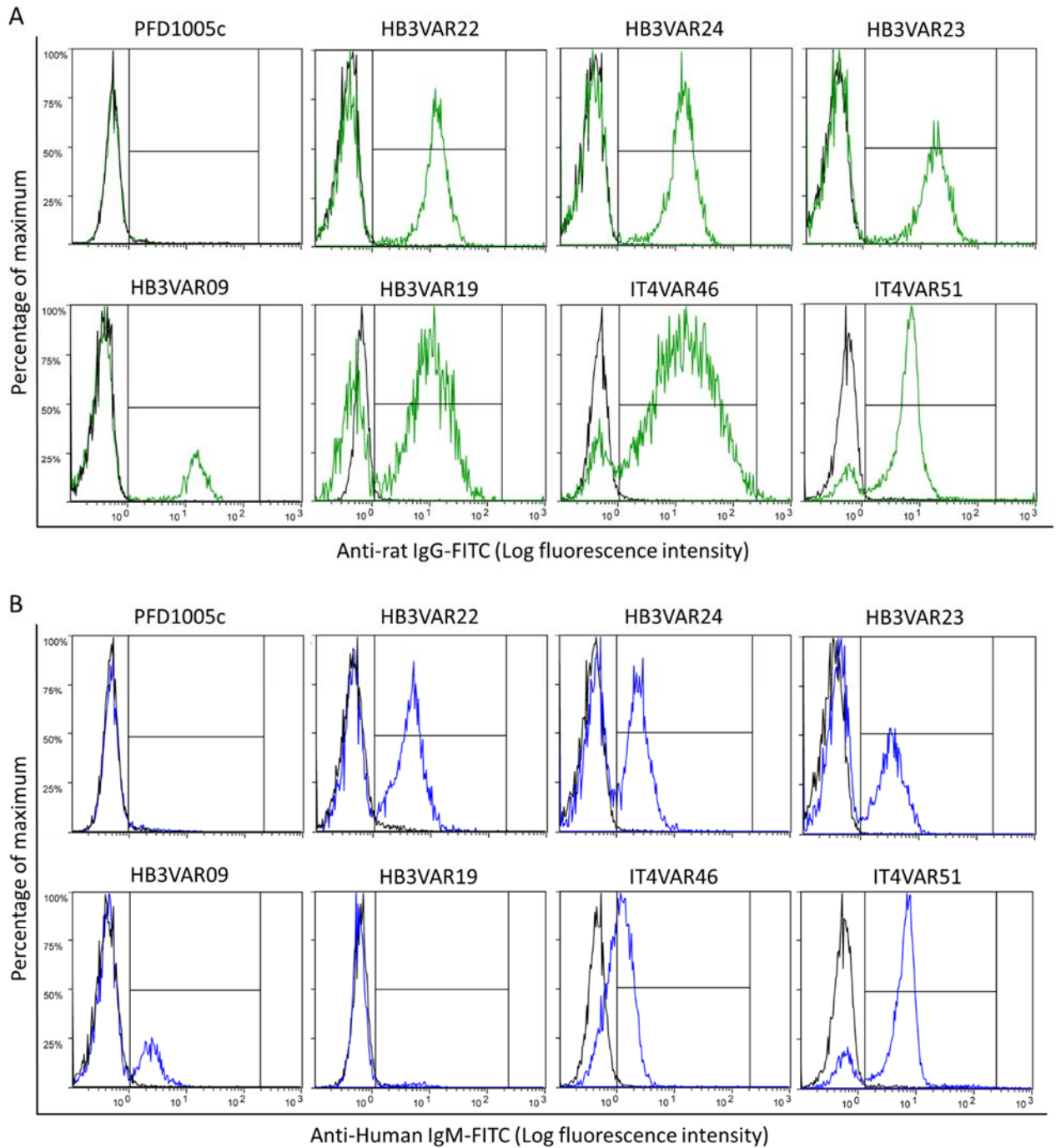


Fig. S7. Non-immune IgM-binding and specific PfEMP1 expression on the surface of infected erythrocytes selected by rat anti-PfEMP1 domain-specific IgG

Data presented as in Fig. 6.

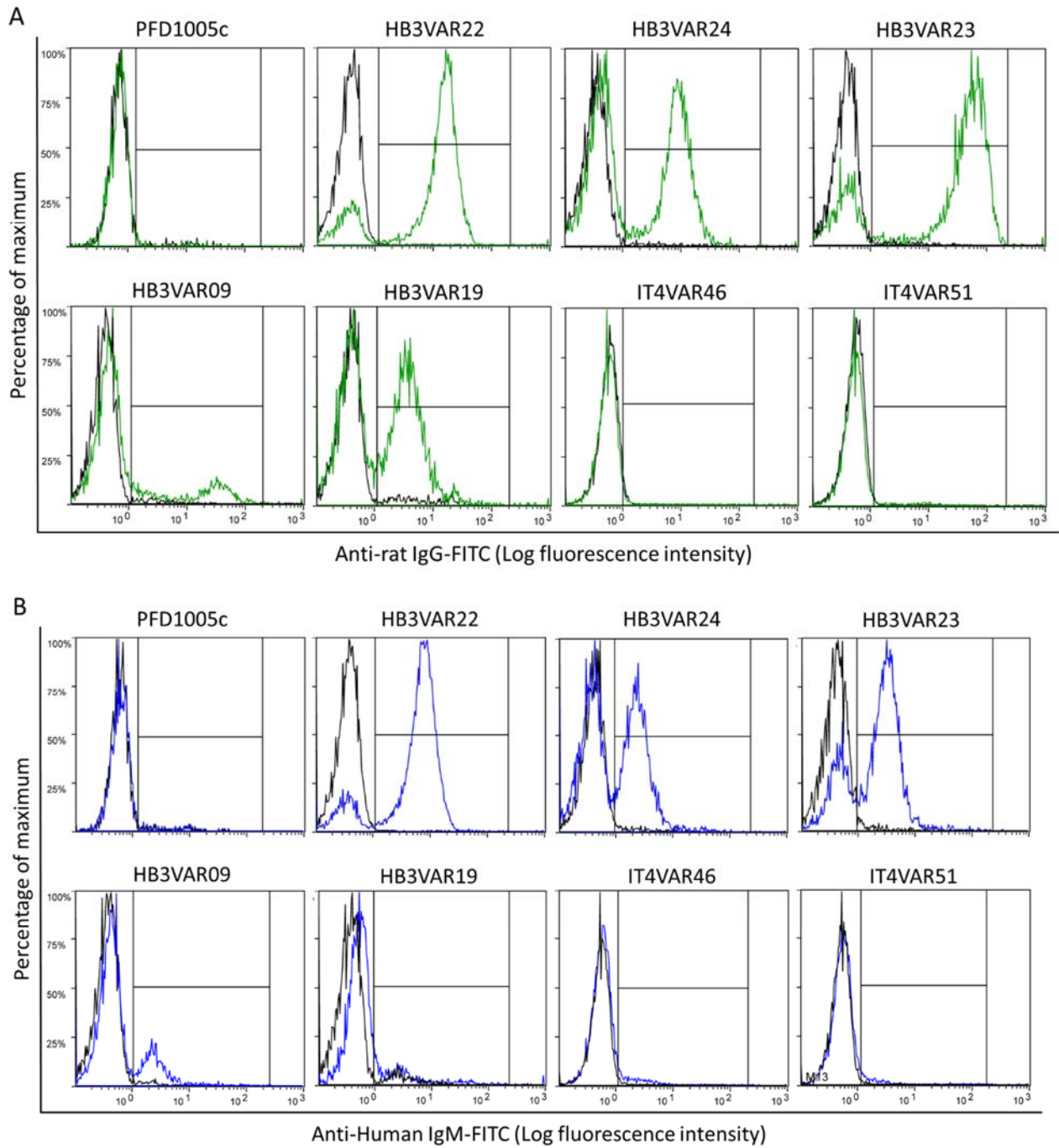


Figure S8. Recombinant protein expression and purification

Coomassie-stained gels (top) and corresponding anti-His immunoblots (bottom) of recombinant proteins from *P. falciparum* 3D7 (A), IT4 (B), and HB3 (C). Arrows indicate the expected size for each construct under reducing conditions (+DTT).

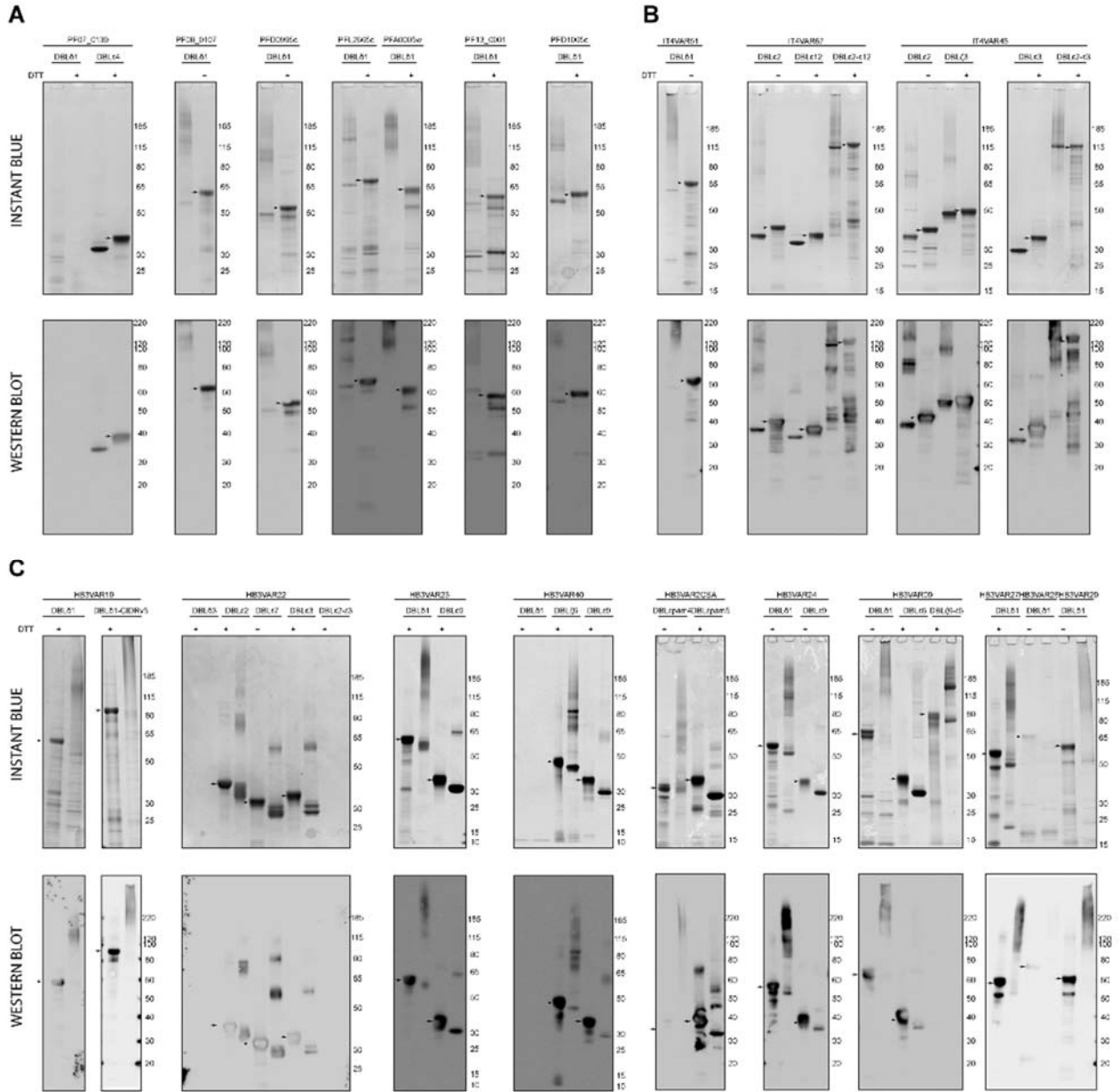


Table S1. var gene transcription in 3D7 sub-clones, sorted according to IE reactivity with non-immune IgM

IgM ⁺ IEs	Sub-clone name	Dominant transcripts					
		Primary (%)		Secondary (%)		Tertiary (%)	
79%	3D7 2.28	<i>pfl0030c</i>	(86)	<i>pf1000c</i>	(2)	<i>pf1005c</i>	(2)
63%	3D7 3.17	<i>pfl0030c</i>	(61)	<i>pf1005c</i>	(6)	<i>pf1000c</i>	(4)
34%	3D7 3.82	<i>pfl0020w</i>	(27)	<i>pfl1955w</i>	(16)	<i>pfl2665c</i>	(16)
25%	3D7 3.30	<i>pf1005c</i>	(15)	<i>pf10005w</i>	(9)	<i>pf1005c</i>	(9)
24%	3D7 4.63	<i>pf1005c</i>	(14)	<i>pf1000c</i>	(9)	<i>pf1015c</i>	(7)
23%	3D7 4.34	<i>pf1005c</i>	(14)	<i>pf1000c</i>	(10)	<i>mal6p1.316</i>	(5)
19%	3D7 2.72	<i>pfl2665c</i>	(63)	<i>pf1005c</i>	(4)	<i>pf1000c</i>	(4)
19%	3D7 3.75	<i>pf1000c</i>	(30)	<i>pf1005c</i>	(22)	<i>pfl0030c</i>	(5)
13%	3D7 2.27	<i>pf1000c</i>	(18)	<i>pf1005c</i>	(13)	<i>pf1015c</i>	(8)
12%	3D7 1.67	<i>pfa0005w</i>	(34)	<i>mal6p1.316</i>	(8)	<i>pf1005c</i>	(7)
10%	3D7 3.50	<i>pf08_0107</i>	(36)	<i>pf1005c</i>	(8)	<i>pf10005w</i>	(4)
8%	3D7 4.59	<i>pf1005c</i>	(19)	<i>pf1000c</i>	(11)	<i>mal6p1.316</i>	(5)
8%	3D7 1.14	<i>pfl0030c</i>	(13)	<i>pf1005c</i>	(11)	<i>pf1000c</i>	(10)
7%	3D7 2.68	<i>pf1005c</i>	(13)	<i>pf1000c</i>	(10)	<i>pfe0005w</i>	(5)
6%	3D7 4.58	<i>pf1005c</i>	(22)	<i>pf1000c</i>	(8)	<i>pf08_0107</i>	(7)
6%	3D7 4.26	<i>pf1015c</i>	(16)	<i>pf1005c</i>	(10)	<i>pfl0020w</i>	(10)
6%	3D7 4.80	<i>pf1005c</i>	(14)	<i>mal6p1.316</i>	(12)	<i>pf1000c</i>	(6)
5%	3D7 3.11	<i>pf1000c</i>	(33)	<i>pf1005c</i>	(23)	<i>pf100625c</i>	(4)
5%	3D7 3.29	<i>pf1005c</i>	(14)	<i>pf1000c</i>	(9)	<i>pf100625c</i>	(6)
1%	3D7 1.43	<i>pf13_0001</i>	(43)	<i>mal6p1.1</i>	(26)	<i>pf100625c</i>	(8)
1%	3D7 3.28	<i>pf1005c</i>	(14)	<i>pf1000c</i>	(9)	<i>pf100625c</i>	(6)
1%	3D7 2.31	<i>pf100625c</i>	(9)	<i>pf1005c</i>	(9)	<i>pf1000c</i>	(9)

Sub-clones with dominant ($\geq 35\%$) transcript are indicated in boldface. Selected sub-clones presented in Fig. 2 and S2 are indicated by shading.

Table S2. var gene transcription in HB3 sub-clones, sorted according to IE reactivity with non-immune IgM

IgM ⁺ IEs	Sub-clone name	Dominant transcripts					
		Primary (%)		Secondary (%)		Tertiary (%)	
72%	HB3 2.83	<i>hb3var2csaA</i>	(87)	<i>hb3var1csa</i>	(5)	<i>hb3var27</i>	(3)
64%	HB3 1.43	<i>hb3var39</i>	(35)	<i>hb3var40</i>	(23)	<i>hb3var27</i>	(8)
60%	HB3 1.26	<i>hb3var40</i>	(75)	<i>hb3var27</i>	(7)	<i>hb3var29</i>	(3)
59%	HB3 1.83	<i>hb3var22</i>	(55)	<i>hb3var27</i>	(14)	<i>hb3var29</i>	(4)
58%	HB3 2.6	<i>hb3var23</i>	(51)	<i>hb3var27</i>	(23)	<i>hb3var26</i>	(3)
55%	HB3 1.12	<i>hb3var19</i>	(58)	Unknown	(18)	<i>hb3var27</i>	(8)
54%	HB3 2.11	<i>hb3var39</i>	(22)	<i>hb3var27</i>	(18)	<i>hb3var40</i>	(17)
54%	HB3 2.41	<i>hb3var39</i>	(20)	<i>hb3var40</i>	(18)	<i>hb3var27</i>	(17)
53%	HB3 1.61	<i>hb3var39</i>	(31)	<i>hb3var40</i>	(17)	<i>hb3var27</i>	(13)
49%	HB3 2.17	<i>hb3var39</i>	(12)	<i>hb3var27</i>	(9)	<i>hb3var09/hb3var47</i>	(8)
49%	HB3 2.68	<i>hb3var39</i>	(22)	<i>hb3var27</i>	(17)	<i>hb3var40</i>	(13)
48%	HB3 1.16	<i>hb3var27</i>	(38)	<i>hb3var23</i>	(17)	<i>hb3var25</i>	(9)
46%	HB3 2.94	<i>hb3var40</i>	(45)	<i>hb3var27</i>	(17)	<i>hb3var29</i>	(7)
43%	HB3 1.75	<i>hb3var39</i>	(20)	<i>hb3var40</i>	(16)	<i>hb3var10</i>	(12)
42%	HB3 1.71	<i>hb3var27</i>	(24)	<i>hb3var39</i>	(16)	<i>hb3var29</i>	(9)
41%	HB3 2.42	<i>hb3var39</i>	(19)	<i>hb3var24</i>	(13)	<i>hb3var27</i>	(13)
40%	HB3 1.74	<i>hb3var27</i>	(30)	<i>hb3var39</i>	(23)	<i>hb3var34</i>	(6)
39%	HB3 2.62	<i>hb3var27</i>	(38)	<i>hb3var39</i>	(22)	<i>hb3var40</i>	(8)
38%	HB3 2.19	<i>hb3var39</i>	(23)	<i>hb3var27</i>	(20)	<i>hb3var29</i>	(11)
37%	HB3 1.40	<i>hb3var27</i>	(24)	<i>hb3var40</i>	(22)	<i>hb3var22</i>	(15)
36%	HB3 2.5	<i>hb3var27</i>	(29)	<i>hb3var29</i>	(19)	<i>hb3var22</i>	(14)
33%	HB3 2.18	<i>hb3var27</i>	(27)	<i>hb3var07</i>	(15)	<i>hb3var23</i>	(13)
27%	HB3 1.37	<i>hb3var27</i>	(36)	<i>hb3var23</i>	(28)	<i>hb3var29</i>	(11)
26%	HB3 2.26	<i>hb3var27</i>	(27)	<i>hb3var39</i>	(17)	<i>hb3var40</i>	(9)
24%	HB3 1.25	<i>hb3var27</i>	(42)	<i>hb3var39</i>	(9)	<i>hb3var40</i>	(6)
22%	HB3 1.84	<i>hb3var24</i>	(54)	<i>hb3var27</i>	(18)	<i>hb3var25</i>	(4)
22%	HB3 2.71	<i>hb3var27</i>	(55)	<i>hb3var09/hb3var47</i>	(18)	<i>hb3var29</i>	(9)
14%	HB3 1.24	<i>hb3var39</i>	(25)	<i>hb3var40</i>	(17)	<i>hb3var29</i>	(16)
6%	HB3 1.54	<i>hb3var29</i>	(45)	<i>hb3var27</i>	(9)	<i>hb3var08</i>	(5)
3%	HB3 1.11	<i>hb3var27</i>	(62)	<i>hb3var29</i>	(16)	<i>hb3var24</i>	(11)
3%	HB3 2.27	<i>hb3var28</i>	(49)	<i>hb3var27</i>	(22)	<i>hb3var29</i>	(12)

Sub-clones with dominant ($\geq 35\%$) transcript are indicated in boldface. Selected sub-clones presented in Fig. 2 and S2 are indicated by shading.

Table S3. var gene transcription in IT4 sub-clones, sorted according to IE reactivity with non-immune IgM

IgM ⁺ IEs	Sub-clone name	Dominant transcripts					
		Primary (%)		Secondary (%)		Tertiary (%)	
82%	IT4 7.3	<i>it4var46</i>	(62)	<i>it4var51</i>	(27)	<i>it4var65</i>	(2)
80%	IT4 8.51	<i>it4var51</i>	(70)	<i>it4var21</i>	(8)	<i>it4var59</i>	(6)
79%	IT4 7.37	<i>it4var51</i>	(67)	<i>it4var34</i>	(13)	<i>it4var59</i>	(4)
78%	IT4 7.72	<i>it4var46</i>	(86)	<i>it4var21</i>	(2)	<i>it4var59/65</i>	(2)
67%	IT4 8.1	<i>it4var67</i>	(76)	<i>it4var21</i>	(6)	<i>it4var59</i>	(3)
63%	IT4 8.7	<i>it4var67</i>	(57)	<i>it4var51</i>	(19)	<i>it4var46</i>	(5)
60%	IT4 8.3	<i>it4var67</i>	(50)	<i>it4var34</i>	(19)	<i>it4var66</i>	(15)
59%	IT4 7.5	<i>it4var67</i>	(81)	<i>it4var59</i>	(7)	<i>it4var21</i>	(7)
50%	IT4 7.4	<i>it4var67</i>	(60)	<i>it4var51</i>	(14)	<i>it4var65</i>	(5)
39%	IT4 8.5	<i>it4var51</i>	(84)	<i>it4var59</i>	(2)	<i>it4var21</i>	(2)
39%	IT4 8.34	<i>it4var46</i>	(91)	<i>it4var34</i>	(1)	<i>it4var21</i>	(1)
37%	IT4 7.38	<i>it4var21</i>	(27)	<i>it4var59</i>	(23)	<i>it4var65</i>	(20)
15%	IT4 8.6	<i>it4var15</i>	(75)	<i>it4var67</i>	(5)	<i>it4var08</i>	(4)

Sub-clones with dominant ($\geq 35\%$) transcript are indicated in boldface. Selected sub-clones presented in Fig. 2 and S2 are indicated by shading.

Table S4. Recombinant proteins used for rat immunization.

PfEMP1 variant	Domain name	Non-immune IgM binding by ELISA
HB3VAR09	DBL ϵ 6	-
HB3VAR19	DBL δ 1-CIDR γ 5	-
HB3VAR22	DBL ϵ 7	+
	DBL ϵ 3	+
HB3VAR23	DBL ϵ 9	-
HB3VAR24	DBL ϵ 9	-
HB3VAR40	DBL ζ 6	+
PFD1005c	DBL δ 1	-
PF07_0139	DBL ϵ 4	+
IT4VAR46	DBL ϵ 3	+
IT4VAR51	DBL δ 1	-
IT4VAR67	DBL ϵ 2	+

Table S5. List of primers used to clone various PfEMP1 domains for recombinant protein expression

	Construct Name*	Cloning Primers	Domain size (aa)
1	PFD0995c DBL δ 1	ATGGATCCTGCAATATAGTAAAAACACTATTTGAGAGC CTAGCTAGCTTAACATGTTTTACAATACGTTGAAAGAGAA	446
2	PFA0005w DBL δ 1	CTAGCTAGCTGCAATATAGTGAACAATGTGTTTACAG CTACTCGAGTTAACATGGAGCACAATAATTTTCATGT	508
3	PFD1005c DBL δ 1	ATGGATCCTGCAATATAGTAAAAACACTATTTGAGAGC CTAGCTAGCTTAACATGTTTTACAATACGTTGAACGAG	496
4	PFL2665c DBL δ 1	ATGGATCCTGCGAAACAGTGGCCACAG CTAGCTAGCTTAACATGGTTTACAATTATCTGCATGTTT	563
5	PF08_0107 DBL δ 1	ATGGATCCTGTGAAATAGTACAAAACTATTTACTAATGG CTAGCTAGCTTAACACGCTTTACAATACGTTGAACG	519
6	PF13_0001 DBL δ 1	CTAGCTAGCTGCAAAACGGTGGCCG CTACTCGAGTTAACATGGATCACAATATTTTGCGT	482
7	PF07_0139 DBL δ 1	GCGGATCCTGCAAAACGGTGGCAGAA CTAGCTAGCTTAACATGGATCACAATATTTTTTATGTCC	487
8	PF07_0139 DBL1 ϵ 4	GCGGATCCATATTTAAAACATGTCCTTATGATAATGATAC CTAGCTAGCTTAGCACTCACATTTACTTTTCAGATTTTTA	322
9	HB3VAR2CSA DBL ϵ pam_D4	ATGGATCCGAACAAGTAAAATATTACAAATATAATAATGCTG CTAGCTAGCTTATTCCGATTCTTTGTTACTTTTACGG	312
10	HB3VAR2CSA DBL ϵ pam_D5	ATGGATCCCATTAGATAGATGCTTTGACGACA CTAGCTAGCTTAACACGGACATTTAGAACAATGTTCC	302
11	HB3VAR40 DBL δ 1	ATGGATCCTGTGAAATAGTTGACGAGCTATTTAAAG CTAGCTAGCTTAACACGCTTTACAATACGTTGAAGG	475
12	HB3VAR40 DBL ζ 6	CTAGCTAGCTGTGTTGAAAGAGCGGCAAAAC CTACTCGAGTTAACATTCACATGCTCTTTCATATTCTTT	417
13	HB3VAR40 DBL ϵ 9	ATGGATCCCATTGGATCAATGTCCTCATACT CTACTCGAGTTAACATTCACATTTTCTTTATTTTAGTATCG	321
14	HB3VAR22 DBL δ 3	ATGGATCCTGCAACACAGTGAAAACCGC CTAGCTAGCTTAACATGTTTTGCAATATTCTGAAGAAC	465

15	HB3VAR22 DBL ϵ 2	ATGGATCCCCGAAAATATTGTGTGGTATTACTAAAAC CTAGCTAGCTTAACATTCACATTCATCTTTAACATCATCA	348
16	HB3VAR22 DBL ϵ 7	ATGGATCCGACCTAGATAAATGTCCAAGTGAAGAA CTAGCTAGCTTAACATTGACATTCAGAACATTTTCTTT	275
17	HB3VAR22 DBL ϵ 3	ATGGATCCCCTTATCTTCGTTGTCCTGATTCAAATTATGT CTAGCTAGCTTAGCAATCACATTTATCTTTATATGTAGGCT	303
18	HB3VAR22 DBL ϵ 2-DBL ϵ 3	ATGGATCCCCGAAAATATTGTGTGGTATTACTAAAAC CTAGCTAGCTTAGCAATCACATTTATCTTTATATGTAGGCT	979
19	HB3VAR23 DBL δ 1	ATGGATCCTGTGACATAGTAAACACACTATTTAGTGACAC CTAGCTAGCTTAACATGCTTTACAATACGTTGAAGGAG	476
20	HB3VAR23 DBL ϵ 9	ATGGATCCCCATTGGATAAATGCCCTAATACT CTAGCTAGCTTAACAGTTACATTTATTTTTCAGGTTGTTATC	321
21	HB3VAR19 DBL δ 1	ATGGATCCTGCAGTATAGTGGCTGACATATTTAAA CTAGCTAGCTTAACAGGTTTCGCAATATTTTGAATG	494
22	HB3VAR19 DBL δ 1-CIDR γ 5	ATGGATCCTGCAGTATAGTGGCTGACATATTTAAA CTAGCTAGCTTAACACGTTTGTGCTTGGTTATCG	769
23	HB3VAR24 DBL δ 1	ATGGATCCTGCGACACAGTGAAAAGCGC CTAGCTAGCTTAACACGTTTTACAATATTCTAAAGGACCA	453
24	HB3VAR24 DBL ϵ 9	ATGGATCCCCATTGGATCAATGTCCTCATACA CTAGCTAGCTTAACAGTTACATTTATTTTTCAGGTTGTTATC	322
25	HB3VAR09 DBL δ 1	ATGGATCCTGTGACATAGTAAAAGAAGTATTTAAAGACAC CTAGCTAGCTTAACACGTTTTACAATAATCTAAGGGGC	531
26	HB3VAR09 DBL ζ 6	CTAGCTAGCTGTGTTGAGAGAATAGCAAAGAATTAA CTACTCGAGTTAACATTCACATACTTTTTCATATTCTTTTG	424
27	HB3VAR09 DBL ϵ 6	CTAGCTAGCCCATTAGATAATTGTCCTAAGGATAATAAAC CTACTCGAGTTAACAAACACATATATTTTAAACATCACTATCG	319
28	HB3VAR29 DBL δ 1	ATGGATCCTGCGAAACAGTGAAAAAAGCAC CTAGCTAGCTTAACATGGATCACAAAGATTTGTATGTTT	490
29	HB3VAR28 DBL δ 1	ATGGATCCTGCCAATAGTAAAAACCGCACTT CTAGCTAGCTTAACATGGATCACAAATGTTTTGTATGTTT	539
30	HB3VAR27 DBL δ 1	GCGGATCCTGTGAAATAGTACAAACACTATTTACTAGTGG	473

		CTAGCTAGCTTAACATGGATTACAATCTTTTGCATGT	
31	IT4VAR46 DBL ϵ 2	GCGCTAGCTCGGATATATTTTGTGATCGTAACAA CTACTCGAGTTAACACGTACACAAATTTTACTTCAT	347
32	IT4VAR46 DBL ζ 3	GCGCTAGCTGTATAGAGAAAGCTGCATATGAACTACA CTACTCGAGTTAACACTTGCATTTTCTTTGTATACTTTAG	431
33	IT4VAR46 DBL ϵ 3	GCGCTAGCCCATTAGATAAATGTCCTGATCAAATAC CTACTCGAGTTAGCAATTACATTTACCTTTAAGTGCTTTAT	309
34	IT4VAR46 DBL ϵ 2-DBL ϵ 3	GCGCTAGCTCGGATATATTTTGTGATCGTAACAA CTACTCGAGTTAGCAATTACATTTACCTTTAAGTGCTTTAT	1104
35	IT4VAR51 DBL δ 1	GCGCTAGCTGTGACATAGTGGCAACACTATTTGA CTACTCGAGTTAACACGTTTTACAATACGTTGAACGA	588
36	IT4VAR67 DBL ϵ 2	ATGGATCCTCGGATAGGTTGTGTGATGATAAGA CTAGCTAGCTTAACACGTACACAACATTTTACTTCAT	345
37	IT4VAR67 DBL ϵ 12	ATGGATCCCCATTAGATGATTGTCCCGACG CTCGCTAGCTTAACAAATATATTTTAATATTTCTTTATATGCGAC	325
38	IT4VAR67 DBL ϵ 2-DBL ϵ 12	ATGGATCCTCGGATAGGTTGTGTGATGATAAGA CTCGCTAGCTTAACAAATATATTTTAATATTTCTTTATATGCGAC	1129

Sequences recognized by the restriction enzymes used for cloning are presented in bold. A stop codon was included in the reverse primer. The enzymes BamHI (GGATCC), NheI (GCTAGC) or XhoI (CTCGAG) were used. *Names, sequences and domain boundaries as reported in the VarDom 1.0 server. (<http://www.cbs.dtu.dk/services/VarDom/>)

Table S6. PfEMP1 constructs from *P. falciparum* 3D7 cloned into pHisAdEx vector*

PfEMP1	Construct name	Cloning primers	Domain size (aa)
1	PFD0020c DBL α 1.2 -CIDR α 1.1 (AA 2-745)	F: CCCGGATCCAGGGGACAGGTTTCATCAACTCCTTCGG R: CCCTCCGGAGCCACCATTATTGTTTGTTCACA	743
2	DBL β 12 (AA 736-1192)	F: CCCGGATCCAGAACCCCTGTGGAACAAACAATAATGG R: CCCGAATTCCTGGTTTTTGTGCCTTCTGCCTAG	456
3	DBL γ 6 (AA 1211-1536)	F: CCCGGATCCAGGGCGGAACAGGTGGAATAGATGG R: CCCGAATTCCTCGGAGTTATTTTTCACTTTTTCC	325
4	DBL γ 11 (AA 1583-1901)	F: CCCGGATCCAGAGCGCAACTGATTATATAGAAAAG R: CCCGAATTCCTTATACTCACAATTTGCACTAGTA	318
5	DBL δ 1-CIDR γ 8 (AA 2119-2894)	F: CCCGGATCCAGAAATATGGCGGAAACAACACTCACGTTT R: CCCGAATTCCTGCTGGGTTTTGGGCACTAAGTA	775
6	PF08_0141 DBL ζ 5 (AA 1700-2077)	F: CCCGGATCCAGCCTTCTATTGAAGAATGTGATATCAA R: CCCGAATTCATCCAATGGATCAAAATCTTTCGCAC	377
7	DBL ϵ 4 (AA 2080-2387)	F: CCCGGATCCAGCCTGTTGATAATAACGAATGTAAGAAG R: CCCTCTAGAAGGTGAATCTTTTTTTTTTTCTTTTTCCG	307
8	PFL0020w DBL α 0.18 CIDR α 5 (AA 2-731)	F: CCCCCTTAAGACATCATGTAGTCCGGAGAATAC	279

		R: CCCGAATTCCCTCTTTGGAGCAATTTTTTTCTTCTCG	
9	DBL β 5_D4 (AA 750-1242)	F: CCCGGATCCAGAATCCGTGTAGCGCCCAACCTGG	492
		R: CCCGAATTCCC GCATCTTTTTTTCTTCTTCCTTC	
10	DBL γ 14 (AA 1255-1592)	F: CCCGGATCCAGGCAACAGATCCTATAGATGGGTG	337
		R: CCCGAATTCCCTGGTTGTCTCGACGCATTTTCC	
11	DBL ζ 5 (AA 1698-2041)	F: CCCGGATCCAGGCAAATAATTTATTGGACACATCTTG	343
		R: CCCGAATTCCATCAGTTTTAACAAATTTAAATCTTCGAC	
12	DBL ϵ 4 (AA 2073-2382)	F: CCCGGATCCAGCCTGTTGACAAGGACGAATGC	309
		R: CCCGAATTCCCTCCATTCCC ACTTTTATCGTATTTTTC	
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13	MAL6P1.4 DBL β 5 (AA 830-1309)	F: CCCGGATCCAGAGTAATCCATGTGCTACTCCTAG	479
		R: CCTCTAGACGGAGGCACTTGTGGCCTAC	
14	DBL γ 13 (AA 1328-1660)	F: CCCGGATCCAGGGTAAGGATGGTGAAACAACAGAAA	332
		R: CCCGAATTCCACTAG AATCTTCATTATGTTGTTGTG	
15	DBL δ 4-CIDR γ 1 (AA 1710-2388)	F: CCCGGATCCAGACTGAAGCTTGTTCAACAAAATATAAAAAT	678
		R: CCCGAATTCCAGAGGTTTCTTCTTCGTC ACTATG	
16	DBL ϵ 2 (AA 2413-2774)	F: CCCGGATCCAGCCCAAACAGATTCCGATATTTTG	361
		R: CCCGAATTCCATCATTAACTTTATCGTCATGTGC	
17	DBL ϵ 7 (AA 2785-3063)	F: CCCGGATCCAGAATAAAAATATATGTAATAAATATAAAAAACGACG	278
		R: CCCGAATTCCCTCTATATCTTTAAACTACATATAC	

18	DBL ϵ 3 (AA 3105-3410)	F: CCCGGATCCAGCCTGAAGACATTGAATGTAGTCAA R: CCCTCTAGAAGGTGTTGTGGGAATCATTTTTTTG	305
19	MAL6P1.316 DBL α 2-CIDR α 1.8 (AA 2-726)#	F: CCCCTCCGGAGCGCCGCAACGTGCCGAAGATTC R: CCCGAATTCCACCACGAGTGGGTATACGAGTGCG	724
20	DBL β 12 (AA 703-1204)	F: CCCGGATCCAGGAAGAAGATGAAAATTGCCCC R: CCCGAATTCCACCTCCAGTGGGTTTTGGTGG	501
21	DBL γ 4 (AA 1265-1603)	F: CCCGGATCCAGTCAAATGGTGGAAAAAATGGAATAAATGG R: CCCTCTAGATGGTTGTCTCGACGCATTTTCCATG	338
22	PF07_0139 DBL α 0.16-CIDR α 3.4 (AA 2-918)	F: CCCGGATCCAGGCGAGGCCATCAGGTAGTGCTGG R: CCCTCTAGAGGCGTCAGTAAGACTGGTTTTGAG	916
23	DBL δ 1-CIDR β 1 (AA 910-1688)	F: CCCGGATCCAGGCACTCAAACCAGTCTTACTGAC R: CCCGAATTCCTTCACTTTCTGGAACTTTCGATTTTTCC	778
24	DBL ϵ 4 (AA 1722-2011)	F: CCCGGATCCAGTATCGAAATAAAAATAACATTGGATGTCC R: CCCGAATTCCTTTATCGTCGTCAATAAAAATTTTGAACG	289
25	PF11_0008 DBL δ 5-CIDR β 4 (AA 1236-1874)	F: CCCGGATCCAGAAATATGGGAAGATGCCACTAGGTTT R: CCCGAATTCATTTTTTACAATGACGTATTTTCAATTTTAAAG	638
26	CIDR β 4 (AA 1922-2420)	F: CCCGGATCCAGAATCCATGTGGCGACAAAAGCGCC R: CCCTCTAGATGCCGGCTCCTCCTCCTCTTCGGC	498
27	PFC0005w DBL α 0.9-CIDR α 2.4 (AA 2-854)	F: CCCGGATCCAGGTTAGGACATTAGATCCTGAGG	852

		R: CCCTCTAGAAGCAGTGTTGTCGCCATTAG	
28	DBL δ 1-CIDR β 1 (AA 848-1685)	F: CCCCTTAAGACTAATGGCGACAACACTGCTC	837
		R: CCCTCTAGATTCCCTCGGATCGACTATCTTCC	
29	PF08_0103 DBL α 0.12-CIDR α 2.2 (AA 2-815)#	F: CCCGGATCCAGTCGACGCTAGGAGGTGGTACTGAC	813
		R: CCCGAATTCCGGCGGCATTCAAGTCGCCTTTGG	
30	DBL δ 1-CIDR β 1 (AA 810-1616)	F: CCCCTTAAGGGCGACTTGAATGCCGCCTGCAC	806
		R: CCCGAATTCCTGATGCCGGTGTACAAGTTTCTCC	
31	PF11_0007 DBL α 0.15-CIDR α 3.2 (AA 2-844)	F: CCCGGATCCAGGAGCCGCATGGAGGTAGCGG	842
		R: CCCGAATTCCTTCCTCCTCCTCCTCCTGCTCTTC	
32	DBL δ 1-CIDR β 1 (AA 908-1700)	F: CCCGGATCCAGAAATACGGTCCAAAGGCACCCAC	792
		R: CCCGAATTCGCCTTTTTCTCCTGCTTTTGGTTC	
33	PFE0005w DBL α 0.11-CIDR α 2.4 (AA 2-793)	F: CCCGGATCCAGGGGCCGCCAGGTATTACTGGTAC	791
		R: CCCGAATTCCTTCTTCCTTTTCTCAGTGGCC	
34	DBL δ 1-CIDR β 1 (AA 862-1706)	F: CCCCTTAAGGACAATCTGAAAGAAGCCTGCAC	844
		R: CCCGAATTCCTCCTTTGTCTTTTCTCCTCCTTG	
35	PF07_0048 DBL α 0.1-CIDR α 3.2 (AA 2-901)	F: CCCGGATCCAGGCGAGGCCAGGTAGCGGTGGTGG	899
		R: CCCGAATTCCTGGGTTCTGAAAGAGTTTAGACAC	
36	DBL δ 1-CIDR β 6 (AA 901-1679)	F: CCCGGATCCAGCCAAACAATTTTTCCGACGCTTGTA	778

			R: CCCGGCGCCTGCTGCTGCTGGTGTACAAGTCTC	
37	PF07_0051	DBL α 0.1-CIDR α 3.1 (AA 2-901)	F: CCCGGATCCAGGCGCCAGGAGGTCGTCAGGGTGAT	899
			R: CCCGAATTCCTGCTTGTTCACGATTTTGCAAACGTC	
38		DBL δ 1-CIDR β 1 (AA 910-1714)	F: CCCGGATCCAGGCCGCCTGTACCCTCAAATATGG	804
			R: CCCGAATTCGGTTCCGCACTAGGTGCTGCTG	

*Sequences recognized by the restriction enzymes used for cloning are presented in bold.

Only primers for constructs presented in Fig. S5 are included in the table. Primers for remaining constructs are available upon request to Andrew Oleinikov.

indicates two exceptions, where construct was cloned into pHisAdEx vector for expression and functional testing.

Reference for Supplementary data

- 1 Fastman, Y., Noble, R., Recker, M. & Dzikowski, R. Erasing the epigenetic memory and beginning to switch - the onset of antigenic switching of *var* genes in *Plasmodium falciparum*. *PLoS One* 7, e34168, doi:10.1371/journal.pone.0034168 (2012).