

Supplementary Materials for

***Plasmodium falciparum* Pf77 and male development gene 1 as vaccine antigens that induce potent transmission-reducing antibodies**

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The PDF file includes:

Figs. S1 to S7
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Other Supplementary Material for this manuscript includes the following:

Data file S1

Supplementary Materials:

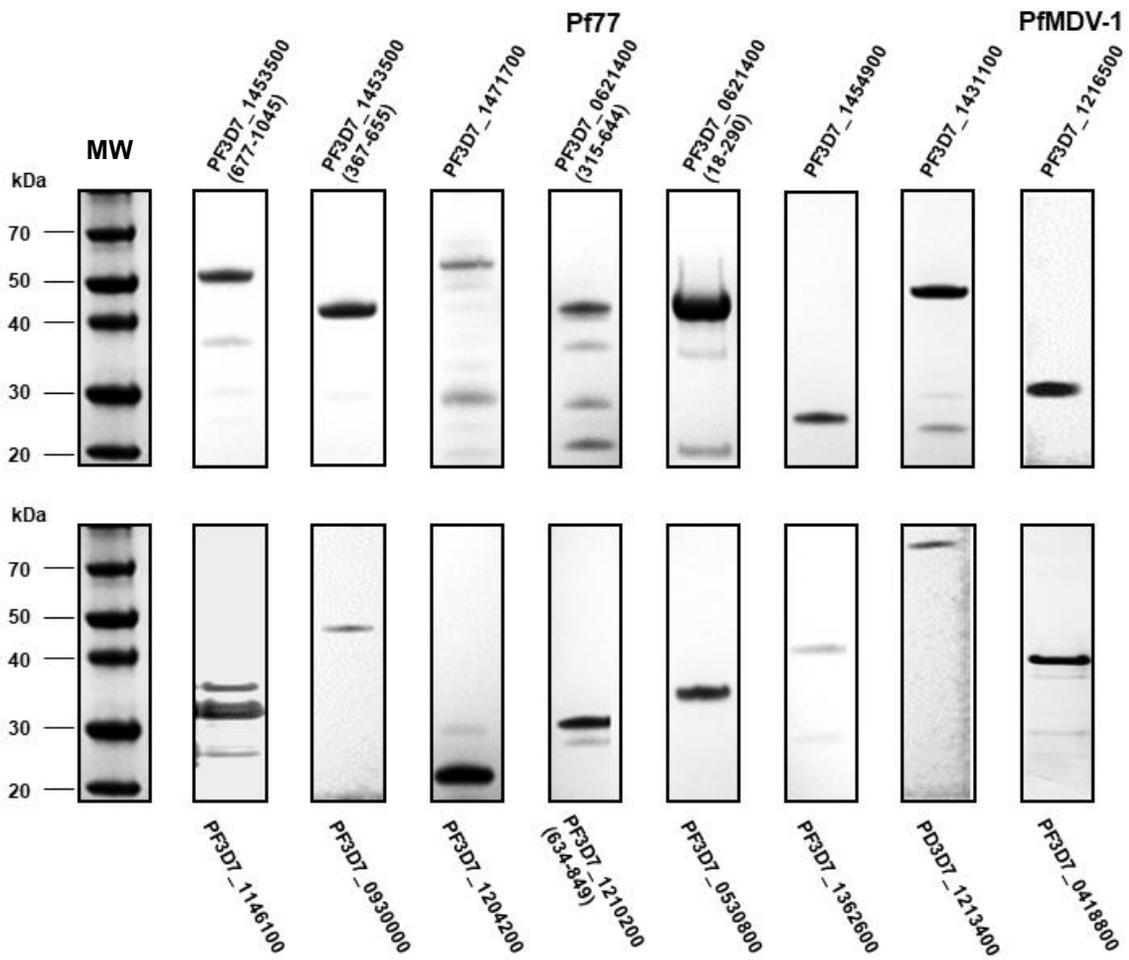


Fig. S1. SDS-PAGE analysis of the recombinant purified 16 *P. falciparum* gametocyte antigen domains expressed in *E. coli*. MW: Molecular weight marker.

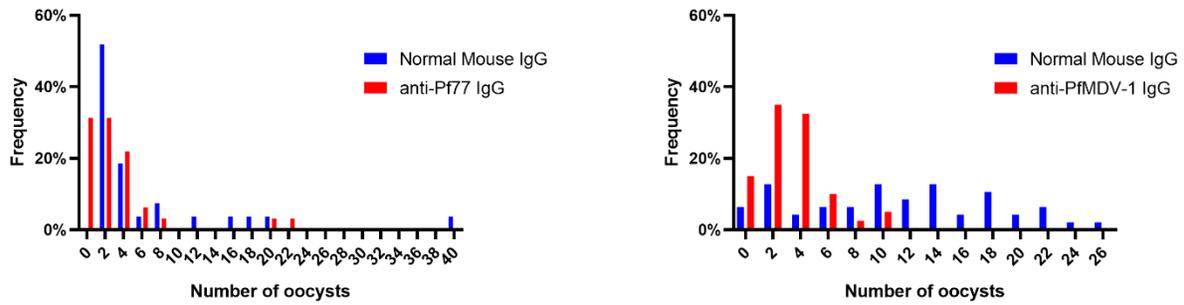


Fig. S2. Effect of anti-Pf77 and anti-PfMDV-1 IgG on oocyst development. *P. falciparum* oocyst development was measured as a reduction in average oocyst burden and proportion of mosquitoes infected in mosquito midgut in the standard membrane feeding assay performed at Johns Hopkins University. Transmission reducing activity of each antigen was assessed in terms of reduction in the total oocyst burden and absence of oocyst in mosquito midguts. Mosquitoes fed on antibodies raised against (A) Pf77 ($n=32$; negative control $n=27$) or (B) PfMDV-1 ($n=40$; negative control $n=47$). Individual oocyst counts acquired from each mosquito were plotted as a histogram.

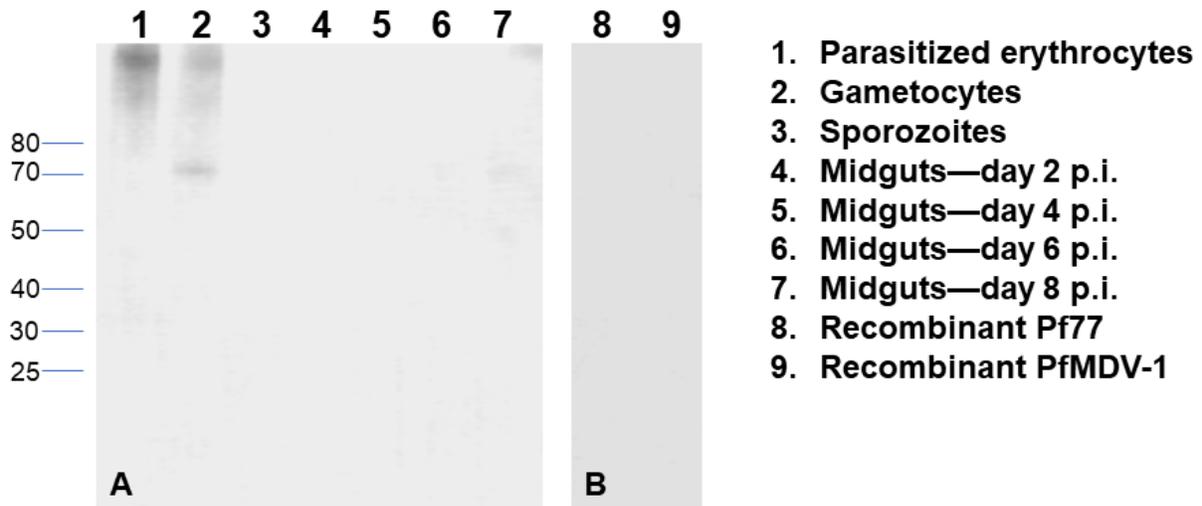


Fig. S3. Western blots demonstrating absence of reactivity of pooled serum samples from non-immunized mice against *P. falciparum* lysates. (A) *P. falciparum* parasite lysates were extracted from (1) 2×10^6 parasitized asexual stage erythrocytes, (2) 1.5×10^6 gametocytes, (3) 1.8×10^6 sporozoites, and (4 to 7) mosquito midguts with approximately 10 oocysts at 2, 4, 6, and 8 days following infection, respectively. Normal mouse serum (1:1000 dilution) exhibited only background reactivity to protein lysates from parasitized erythrocytes and gametocytes and no reactivity to any of the other stages. (B) A second gel was loaded with 100 ng of recombinantly expressed (8) Pf77 and (9) PMDV-1. Normal mouse serum exhibited no reactivity to either recombinant protein.

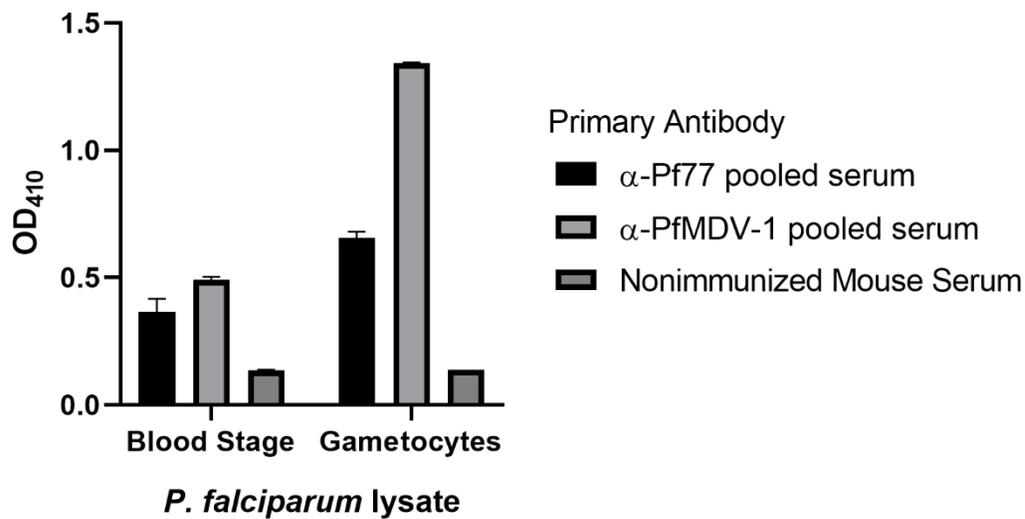
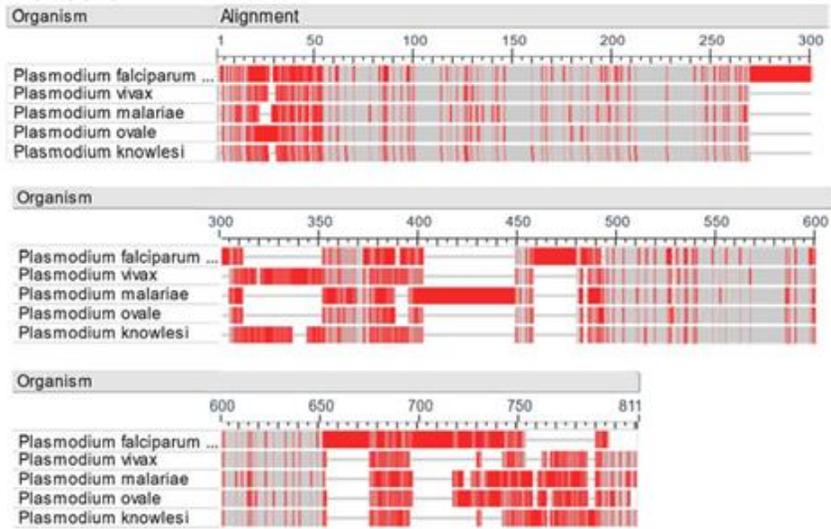


Fig. S4. ELISA IgG reactivity of pooled mouse sera from mice immunized with recombinant Pf77 or PfMDV-1. ELISA plates were coated with 200 ng/well of protein lysate from *P. falciparum* schizonts or gametocytes. Wells were incubated with 1:50 dilutions of pooled mouse sera either from mice immunized with recombinant Pf77 or recombinant PfMDV-1. Pooled sera from nonimmunized mice served as control. Based on the higher ELISA reactivity in prior assays for reactivity of antibody targeting PfMDV-1 towards gametocytes, wells were coated with 100 ng of parasite lysate and incubated with 1:1000 dilutions of pooled immunized mouse serum. Following incubation with goat anti-mouse IgG HRP-conjugated antibody, antigen-antibody reactivity was detected by development with ABTS [2,2'-azino-di-(3-ethylbenzthiazoline sulfonic acid)] substrate for 15 minutes. ELISA IgG reactivity against protein lysates from *P. falciparum* schizonts (Pf77: $P = 0.0242$; PfMDV-1: $P = 0.00053$) and gametocytes (Pf77: $P = 0.00107$; PfMDV-1: $P = 0.000006$) was observed in pooled serum samples from recombinant Pf77- or PfMDV-1-immunized mice.

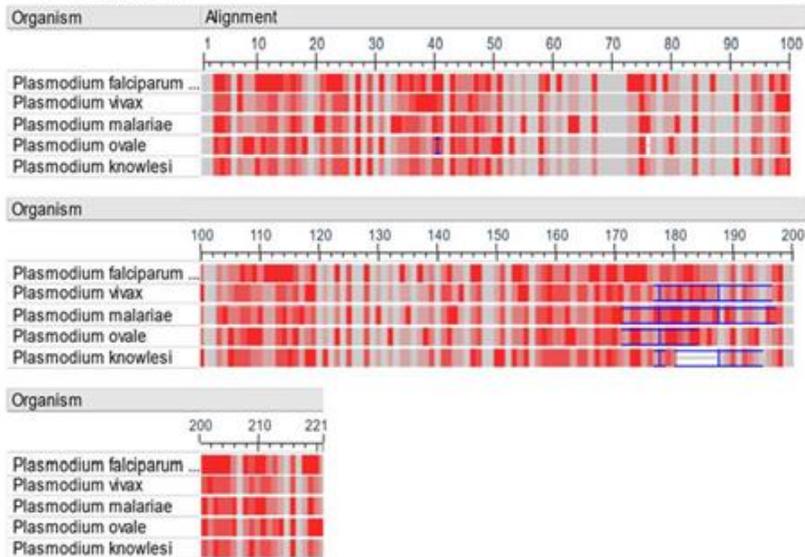
A. Pf77



B.

Species	Accession No.	Length (aa)	Identity (%)	Coverage (%)
<i>P. falciparum</i>	XP_966198.1	664	100.00	100.00
<i>P. vivax</i>	KM279839.1	597	51.92	79.97
<i>P. malariae</i>	XP_028062008.1	619	49.93	81.33
<i>P. ovale</i>	SBS85303.1	583	57.64	83.28
<i>P. knowlesi</i>	OTN64542.1	604	51.29	79.97

C. PfMDV-1



D.

Species	Accession No.	Length (aa)	Identity (%)	Coverage (%)
<i>P. falciparum</i>	XP_001350568.1	221	100.00	100.00
<i>P. vivax</i>	SC075418.1	268	35.42	100.00
<i>P. malariae</i>	SCP03455.1	329	32.83	100.00
<i>P. ovale</i>	SCQ16983.1	241	43.39	99.55
<i>P. knowlesi</i>	OTN64272.1	248	39.22	96.83

Fig. S5. Sequence alignment of orthologs of *P. falciparum* 3D7 proteins Pf77 and PfMDV-1.

Orthologous sequences to (A) Pf77 and (C) PfMDV-1 were searched for *P. vivax*, *P. malariae*, *P. ovale* and *P. knowlesi* by using the NCBI BLASTp tool. Sequences of these human *Plasmodium* species isolates were aligned using the NCBI COBALT tool and anchored to the sequence derived from the *P. falciparum* 3D7 reference genome. Sequence identity and coverage of the homologs from each *Plasmodium* species compared to the *P. falciparum* 3D7 reference genome are shown in (B) and (D) for Pf77 and PfMDV-1, respectively.

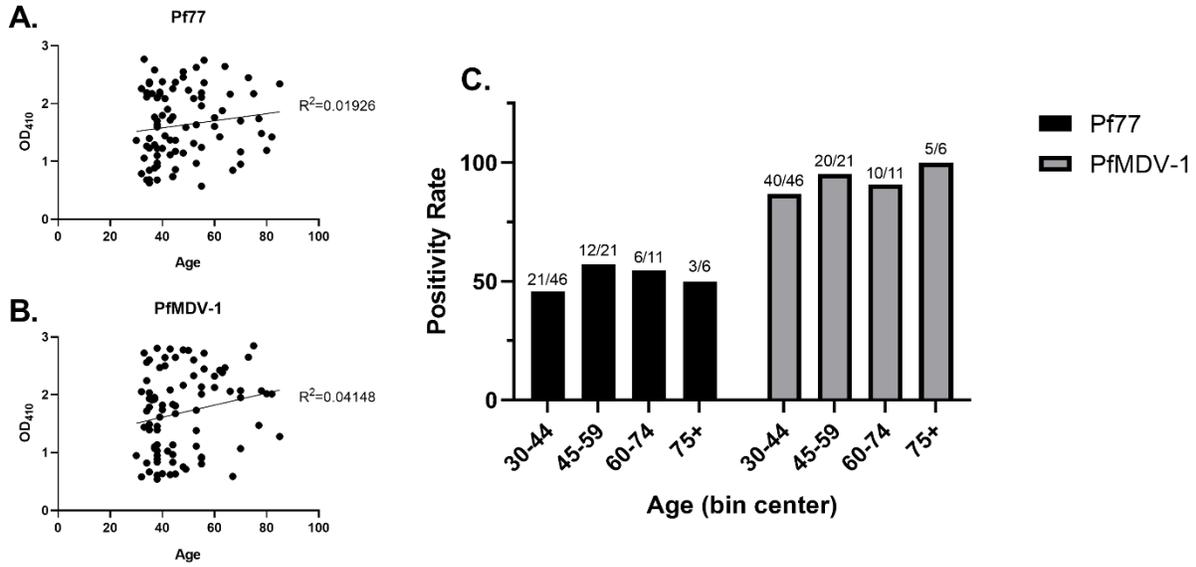


Fig. S6. Seroreactivity to Pf77 and PfMDV-1 among Ghanaian adults compared to age. (A and B) IgG reactivity (OD₄₁₀) towards recombinant Pf77 (A) and recombinant PfMDV-1 (B) was plotted against age in Ghanaian serum samples. The coefficient of determination (R^2) is shown. (C) ELISA positivity rate was plotted based on age of the individual.

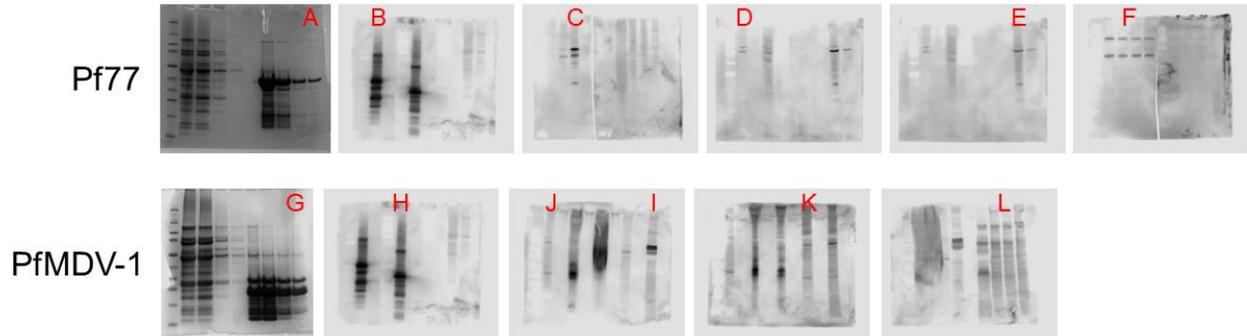


Fig. S7. Raw SDS-PAGE and Western blot images of recombinant Pf77 and PfMDV-1. Raw data showing the original gels and blots from which lanes were used to construct figure 3 in the main article. *P. falciparum* proteins Pf77 and PfMDV-1 were expressed recombinantly. The purity and identity of each recombinant protein was confirmed by SDS-PAGE analysis (**A, G**). IgG was purified from pooled antisera from five mice following immunization with recombinant protein and reactivity of the antisera to the recombinant protein was confirmed by Western blot (**B, H**). The IgG was used to detect the presence of either antigen in protein lysates from each stage of the *P. falciparum* life cycle by Western blot. The equivalent of 4.5×10^5 sporozoites (**C, I**), 1.875×10^6 parasitized erythrocytes (**D, J**), 1.2×10^6 gametocytes (**E, K**), and 10 oocysts (**F, L**) were loaded into each well. Following optimization of protein, primary antibody, and secondary antibody concentrations, representative lanes from SDS-PAGE gels and Western blots are presented in Fig. 3B. The lanes included in Fig. 3B are indicated in the panels (A to L).

Table S1. *Plasmodium falciparum* genes selected for recombinant expression in *E. coli*, immunization of BALB/c mice, and evaluation by standard membrane feeding assay. Based on the results of microarray analysis, 56 genes were identified as being transcriptionally overexpressed in gametocytes as compared to asynchronous blood stage parasites. Of these genes, 16 constructs from 13 genes from the dataset plus one additional gene (Pf3D7_120-4200) were recombinantly expressed in *E. coli*. Female BALB/c mice (6-8 weeks old) were immunized by four subcutaneous injections at four-week intervals with an emulsion of 20 µg recombinant protein along with either a combination of Montanide and CpG (100 µL and 25 µg in 100 µL PBS, respectively) or a series including Complete Freund's Adjuvant (primary immunization) and Incomplete Freund's Adjuvant (three boosts). Five mice were immunized per group; ultimately, 39 immunizations were performed, with serum collected from 195 experimental mice. Serum was collected two weeks following the four immunizations and IgG was purified. Initial screening for transmission reducing activity (TRA) by the standard membrane feeding assay (SMFA) was performed at the Johns Hopkins Malaria Research Institute (JHMRI). Subsequent screenings of antigens displaying highest TRA in the assays performed at JHMRI were sent to the National Institutes of Health (NIH) laboratory for further evaluation.

Gene	PlasmoDB ID	Antigenic Domain	Primers
conserved protein, unknown function	PF3D7_1362600	1-267	5'-AGAAAGTTTAGATGAGAGATGGA-3' 5'-TAATTTGATCCTCTTAAATATTTCTTCT-3'
conserved protein, unknown function	PF3D7_1146100	1-175	5'-AAAGTGTTTAATTTTTGTTTCTCC-3' 5'-TGATTCAGTCTTTATAAGGACG-3'
NAD(P) transhydrogenase	PF3D7_1453500	367-655	5'-AGATTATGAAAATATGGGAGAATC-3' 5'-GACAATAGATAAATTAACATCACG-3'
NAD(P)	PF3D7_1453500	677-1045	5'-AGATATAGGTACCAATATATTAATGC-3'

transhydrogenase			5'–TTCCGATATTGGTTCAATAAGTTG–3'
conserved			
Plasmodium			
protein,	PF3D7_1471700	15–527	5'–AGATATAAAAGGGAACGATATTTATGATAAC–3'
unknown			5'–CTTTTTATTCTTAATATCAGTCCT–3'
function			
Pf77 protein	PF3D7_0621400	18 - 290	5'–AAAAGAAAAAGAGGACGATGGT–3'
			5'–ATGCATATTACTATCATTATATAC–3'
Pf77 protein	PF3D7_0621400	315 - 664	5'–AGACGAATCTGTAAAGAAATCCCT–3'
			5'–ATTAATATCTTCATTTTGCTTGC–3'
conserved			
Plasmodium			
protein,	PF3D7_1204200	155 - 292	5'–AAACATGACGAATGTTCAAGGGA–3'
unknown			5'–TTCCATATCGGAATATTTTTCAC–3'
function			
procollagen			
lysine 5-			5'–ATCCAAAGATGAAAAAAAAATAATAG–3'
dioxygenase,	PF3D7_0930000	1 - 463	5'–AGATGTATACTTATTTTCAAACCCA–3'
putative			
zinc finger			5'–AGATAATAAAAATGTTCATTAGAG–3'
protein, putative	PF3D7_1210200	634 - 980	5'–TTTCCATTAGATGTTATAGAT–3'
conserved			
protein,	PF3D7_1454900	3 - 154	5'–ACATCCCTTTAATTTTGTGCCT–3'

unknown			5'-CATAATATTTTTATTTATATTGGTCA-3'
function			
PhIL1			
interacting	PF3D7_1431100	1 - 340	5'-AGATAAAGATAAGGATAAAAGCAAAAAC-3'
protein PIP2			5'-TGTTTCCTTGAATCTATGAAATTC-3'
MOLO1			
domain-			5'-AGTCCCTATAGAAAATGCTCCT-3'
containing	PF3D7_0418800	21 - 230	5'-TTGAGCAGTTTGTGTTGGTGGGA-3'
protein, putative			
male			
development	PF3D7_1216500	30 - 221	5'-AAACATAGGAAAACCTCAGGTTAAC-3'
gene 1			5'-ATCACTATCACTGTGTGTTTTTATATC-3'
kelch domain-			
containing	PF3D7_1213400	1 - 576	5'-ATCAGATATTCAGATTTCTCTG-3'
protein			5'-TTTATTTTCCATATTTATTTGTGGTTCCT-3'
CPW-WPC			
family protein	PF3D7_0530800	25 - 254	5'-AGATAAGAAAGAGGAACTTTTTTCTGAC-3'
			5'-GTGATAATTTTCTTCAACCTC-3'

Table S2. Confirmation of the identity of potent transmission reducing antigens by mass spectrometry. The amino acid sequence identities of recombinant Pf77 and PfMDV-1 used to immunize mice were confirmed by mass spectrometry. Recombinant proteins were run on an SDS-PAGE gel and the contents of the largest band were digested overnight with trypsin. The resulting proteolytic peptides were analyzed by liquid chromatography-mass spectrometry using a Thermo Fisher Ultimate LC and Fusion Orbitrap MS.

Accession	Description	Coverage	# Peptides	# PSMs	# Unique Peptides	# Protein Groups	# AAs	MW [kDa]	calc. pl	Protein FDR Confidence Sequents HT	Exp. q-value Sequents HT	Score Sequents HT	# Peptides Sequents HT
C6KT52	Pf77 protein OS=Plasmodium falciparum (isolate 3D7) OX=36329 GN=PF3D7_0621400 PE=4 SV=1	16.56626506	14	355	14	1	664	77.406	5.22	High	0	1078.809012	14
		Confidence	Sequence	Modifications	Contaminant	# Protein Groups	# Proteins	# PSMs	Master Protein Accessions	# Missed Cleavages	Theo. MH+ [Da]	XCORR Sequents HT	Confidence Sequents HT
		High	NNNIFGYDINMNGNHR	2xDeamidated [N]; 1xOxidation [M11]	FALSE	1	1	17	C6KT52	0	1910.8086	5.933856487	High
		High	NNNIFGYDINMNGNHR	1xDeamidated [N]; 1xOxidation [M11]	FALSE	1	1	141	C6KT52	0	1909.824584	5.705883503	High
		High	EKEDDGLNTGYINK		FALSE	1	1	35	C6KT52	1	1595.754756	5.405125618	High
		High	HDVPTVELLYKEK		FALSE	1	1	13	C6KT52	1	1570.847534	5.368060112	High
		High	INEVEKEIDVVK	1xDeamidated [N2]	FALSE	1	1	5	C6KT52	1	1415.762802	5.308003902	High
		High	INEVEKEIDVVK		FALSE	1	1	39	C6KT52	1	1414.778786	5.090067387	High
		High	EKEDDGLNTGYINK	1xDeamidated [N8]	FALSE	1	1	4	C6KT52	1	1596.738772	4.884541512	High
		High	NNNIFGYDINMNGNHR	1xOxidation [M11]	FALSE	1	1	18	C6KT52	0	1908.840568	4.592949867	High
		High	INEVEKEIDVVKYK		FALSE	1	1	4	C6KT52	2	1705.937077	4.553369522	High
		High	EDDGLNTGYINK		FALSE	1	1	13	C6KT52	0	1338.6172	4.366192818	High
		High	KEKEDDGLNTGYINK		FALSE	1	1	1	C6KT52	2	1723.849719	4.200821877	High
		High	FVEKEIIVDIKEK		FALSE	1	1	1	C6KT52	2	1589.914886	4.192780018	High
		High	YNEIFYNKDGGK	1xDeamidated [N7]	FALSE	1	1	9	C6KT52	1	1391.647772	4.160851479	High
		High	EKYQDVPVCK	1xCarbamido methyl [C9]	FALSE	1	1	8	C6KT52	1	1265.619449	4.066853523	High
		High	YNEIFYNKDGGK		FALSE	1	1	12	C6KT52	1	1390.663756	3.881328821	High
		High	HDVPTVELLYK		FALSE	1	1	21	C6KT52	0	1313.709978	3.673598528	High
		High	IIQVEIPQIK	1xDeamidated [Q3]	FALSE	1	1	6	C6KT52	0	1181.714002	3.425982952	High
		High	IIQVEIPQIK		FALSE	1	1	2	C6KT52	0	1180.729986	3.362946033	High
		High	KVNIPQEK		FALSE	1	1	1	C6KT52	1	955.5571067	3.289881229	High
		High	YNEIFYNFK		FALSE	1	1	5	C6KT52	0	1090.520387	3.191084146	High

Accession	Description	Coverage	# Peptides	# PSMs	# Unique Peptides	# Protein Groups	# AAs	MW [kDa]	calc. pI	Protein FDR Confidence Sequence HT	Exp. q-value Sequence HT	Score Sequence HT	# Peptides Sequence HT
Q8ISP2	Male development gene 1 OS=Plasmodium falciparum (isolate 3D7) OX=36329 GN=PF3D7_1 216500 PE=4 SV=1	57.46606	17	660	17	1	221	25.742	4.77	High	0	2398.402315	17
Checked	Confidence	Sequence	Modifications	Contaminant	# Protein Groups	# Proteins	# PSMs	Master Protein Accessions	# Missed Cleavages	Theo. MH+ [Da]	XCorr Sequence HT	Confidence Sequence HT	
FALSE	High	KSFETFEQALHNK		FALSE	1	1	82	Q8ISP2	1	1578.791082	5.913624763	High	
FALSE	High	TKNPDLDEQTKDIK		FALSE	1	1	52	Q8ISP2	2	1644.843905	5.89708519	High	
FALSE	High	NVNSNEKANNLSTEW NR	2xDeamidate d [N]	FALSE	1	1	15	Q8ISP2	1	2078.937365	5.426760674	High	
FALSE	High	IQNKIGEYKDEK		FALSE	1	1	5	Q8ISP2	2	1577.853348	5.378252506	High	
FALSE	High	HYVKDVSEYIGAK		FALSE	1	1	13	Q8ISP2	1	1508.774369	5.202528	High	
FALSE	High	NPDLEQTKDIK		FALSE	1	1	29	Q8ISP2	1	1415.701264	5.180547237	High	
FALSE	High	NVNSNEKANNLSTEW NR	1xDeamidate d [N]	FALSE	1	1	68	Q8ISP2	1	2077.953349	5.14323616	High	
FALSE	High	STDNFVPFEENK	1xDeamidate d [N]	FALSE	1	1	33	Q8ISP2	0	1541.675444	4.86692524	High	
FALSE	High	AGDLLDENLESTLKLLE R		FALSE	1	1	5	Q8ISP2	0	2126.133939	4.857997894	High	
FALSE	High	NGYLQSHGNLEEVK		FALSE	1	1	15	Q8ISP2	0	1587.77616	4.789003372	High	
FALSE	High	NGYLQSHGNLEEVK	1xDeamidate d [Q/N]	FALSE	1	1	28	Q8ISP2	0	1588.760176	4.660150528	High	
FALSE	High	TKNPDLDEQTK		FALSE	1	1	27	Q8ISP2	1	1288.637936	4.598171234	High	
FALSE	High	NGYLQSHGNLEEVKDE LANELANK		FALSE	1	1	3	Q8ISP2	1	2685.311462	4.503121376	High	
FALSE	High	STDNFVPFEENK		FALSE	1	1	142	Q8ISP2	0	1540.691428	4.484462738	High	
FALSE	High	NVNSNEKANNLSTEW NR		FALSE	1	1	6	Q8ISP2	1	2076.969333	4.480533123	High	
FALSE	High	SFETFEQALHNK		FALSE	1	1	39	Q8ISP2	0	1450.696119	4.318011761	High	
FALSE	High	ANNLSTEW NR		FALSE	1	1	30	Q8ISP2	0	1291.602553	4.06025219	High	
FALSE	High	DELANELANK		FALSE	1	1	13	Q8ISP2	0	1116.553143	4.054634094	High	
FALSE	High	ANNLSTEW NR	1xDeamidate d [N]	FALSE	1	1	41	Q8ISP2	0	1292.586569	3.953553677	High	
FALSE	High	IGEYKDEK		FALSE	1	1	4	Q8ISP2	1	1094.572816	3.612656116	High	
FALSE	High	NPDLEQTK		FALSE	1	1	2	Q8ISP2	0	1059.495294	3.58833766	High	
FALSE	High	DVSEYIGAK		FALSE	1	1	8	Q8ISP2	0	981.4887523	3.239928722	High	

Table S3. Schedule and results of the standard membrane feeding assay performed at Johns Hopkins Malaria Research Institute. Serum samples collected from mice immunized with each listed antigen was purified and tested at 500µg/mL; negative controls contained purified IgG from naïve mice (tested at 750µg/mL), whereas positive controls used monoclonal antibody 4B7 against Pfs25 known to possess reliable transmission reducing activity (tested at 100µg/mL).

^aThe bolded antigens were those that induced IgG with notable transmission reducing activity (%TRA).

^bTransmission-reducing activity was quantified by comparing each experimental IgG sample to the negative control from the same SMFA.

Date of SMFA	Antigen^a	Adjuvant	Mean Oocyst	%TRA^b
12/17/201				
8				
	Neg Control		25.35 (0-59, n26)	
	Pos Control		1.57 (0-7, n39)	93.78
	PF3D7_1454900	CFA	34.14 (0-79, n36)	-34.67
	PF3D7_1471700	CFA	28.20 (0-58, n30)	-11.24
	PF3D7_0621400	CFA	25.78 (0-47, n37)	-1.69
	(18-290)			

PF3D7_1431100	CFA	23.82 (0-51, n22)	6.03
PF3D7_1216500	CFA	21.67 (0-53, n46)	14.51
PF3D7_1453500 (357-655)	CFA	6.42 (0-31, n19)	74.67

1/7/2019

Neg Control		17.44 (0-40, n25)	
Pos Control		0.91 (0-5, n24)	94.74
PF3D7_1454900	Montanide/CpG	17.82 (0-49, n36)	-2.17
PF3D7_1216500	Montanide/CpG	17.14 (0-52, n37)	1.72
PF3D7_1453500 (367-655)	CFA	16.52 (0-36, n25)	5.27
PF3D7_1431100	Montanide/CpG	15.00 (0-36, n36)	13.99
PF3D7_0621400 (18-290)	Montanide/CpG	14.45 (0-41, n22)	17.14
PF3D7_1471700	Montanide/CpG	9.84 (0-33, n32)	43.55
PF3D7_1453500 (367-655)	Montanide/Cp G	8.42 (0-18, n21)	51.66

2/15/2019

Neg Control		5.25 (0-20, n28)	
Pos Control		0.32 (0-1, n31)	93.85
PF3D7_0530800	CFA	3.48 (0-20, n39)	33.58
PF3D7_1216500	CFA	2.87 (0-13, n40)	45.23
PF3D7_1210200	CFA	1.76 (0-11),	66.47
(634-849)		n25)	
PF3D7_1213400	CFA	1.58 (0-16, n29)	69.79

2/22/2019

Neg Control		24.05 (0-56, n39)	
Pos Control		0.53 (0-2, n30)	97.78
PF3D7_1204200	CFA	17.84 (0-33, n38)	25.82
PF3D7_1453500	CFA	16.18 (0-34, n51)	32.72
PF3D7_0621400	CFA	14.87 (0-27,	38.17
(315-664)		n31)	
PF3D7_0930000	CFA	14.48 (0-41, n25)	39.79
PF3D7_1146100	CFA	12.98 (0-26,	46.02
		n42)	

2/28/2019

Neg Control		5.96 (0-39, n27)	
Pos Control		1.13 (0-6, n38)	81.01
PF3D7_1204200	Montanide/CpG	9.30 (0-41, n39)	-56.09
PF3D7_1453500	Montanide/CpG	8.67 (0-33, n40)	-45.48
(677–1045)			
PF3D7_1146100	Montanide/CpG	8.48 (0-41, n25)	-42.21
PF3D7_0930000	Montanide/Cp	4.42 (0-29, n40)	25.79
	G		
PF3D7_0621400	Montanide/Cp	2.84 (0-21, n32)	52.3
(315–644)	G		

3/7/2019

Neg Control		10.70 (0-25, n47)	
Pos Control		5.97 (0-14, n38)	44.17
PF3D7_1362600	Montanide/CpG	6.52 (0-28, n36)	38.99
PF3D7_1210200	Montanide/Cp	4.55 (0-21, n49)	57.46
(634–849)	G		
PF3D7_1213400	Montanide/Cp	2.95 (0-13, n45)	72.37
	G		
PF3D7_1216500	Montanide/Cp	2.77 (0-9, n40)	74.06
	G		
PF3D7_0530800	Montanide/Cp	2.21 (0-13, n49)	79.32
	G		

3/22/2019

Neg Control		38.66 (0-93, n27)	
Pos Control		2.19 (0-13, n26)	94.32
	CFA	43.20 (0-83, n31)	-11.76
PF3D7_0827200			
PF3D7_0418800	CFA	34.06 (0-65, n29)	11.87
(21-230)			
PF3D7_0621400	CFA	30.12 (0-71, n24)	22.07
(315-644)			

Table S4. Results of standard membrane feeding assay performed at National Institutes of Health using sera with putative transmission-reducing activity. Experimental sera were

spiked into blood culture at a final concentration of 750 µg/mL. Otherwise, SMFA was performed as described in Supplemental Table 2. All experimental sera were derived from BALB/c mice immunized with the listed antigen and Montanide/CpG as an adjuvant unless otherwise noted. All antigens were produced recombinantly in *E. coli*.

^aAssay #1 included all 10 putative transmission-blocking sera as identified by SMFA at JHMRI.

^b Complete Freund’s Adjuvant (CFA) was used as an adjuvant to elicit indicated serum samples.

PF3D7_0621400 (315-664) [Pf77], PF3D7_1210200 (634-849) [zinc finger protein, putative], and PF3D7_1216500 [PfMDV-1] yielded significant %TRA (>80%) and were re-evaluated immediately in Assay #2. PF3D7_0621400 (315-664) [Pf77] and PF3D7_1216500 [PfMDV-1] once again displayed transmission-blocking potential. The remaining antigens were screened once more in Assay #3. PF3D7_0930000 exhibited >80% TRA in Assay #3. PF3D7_0621400 (315-664) [Pf77] and PF3D7_1216500 [PfMDV-1] were selected for further evaluation due to their consistent reduction of oocyst formation in SMFA, but PF3D7_1210200 (634-849) [zinc finger protein, putative] and PF3D7_0930000 [procollagen lysine 5-dioxygenase, putative] should be noted for future experiments.

^cNumber of infected mosquitoes out of 20 mosquitoes dissected.

Name	Mean oocyst	%TRA	(95%CI)	p-value	Infect mosq ^c
Assay #1^a					
Control	2.9				
PF3D7_0621400 (315-664)	0.1	96.6	(90 to 99)	0.001	2

PF3D7_1453500 (367-655)	2.7	8.6	(-117 to 65)	0.829	11
PF3D7_1210200 (634-849) ^b	1.4	53.4	(-40 to 89)	0.145	11
PF3D7_1210200 (634-849)	0.6	81.0	(42 to 96)	0.004	6
PF3D7_0530800	0.9	70.7	(-33 to 95)	0.081	4
PF3D7_1213400 ^b	1.0	65.5	(-13 to 93)	0.071	7
PF3D7_1213400	2.4	17.2	(-167 to 76)	0.679	8
PF3D7_1146100 ^b	1.8	39.7	(-39 to 73)	0.237	6
PF3D7_1216500	0.3	89.7	(69 to 98)	0.001	3
PF3D7_0930000	1.1	62.1	(4 to 87)	0.036	10

Assay #2

Control	11.8				
PF3D7_0621400 (315-664)	1.6	86.9	(71 to 95)	0.001	7
PF3D7_1210200 (634-849)	7.7	35.2	(-44 to 72)	0.284	13
PF3D7_1216500	2.8	76.3	(17 to 96)	0.014	8

Assay #3

Control	6.3				
PF3D7_1453500 (367-655)	5.2	17.6	(-83 to 64)	0.640	12
PF3D7_1210200 (634-849) ^b	3.6	42.4	(-33 to 75)	0.194	13
PF3D7_0530800	2.3	64.0	(4 to 88)	0.034	10
PF3D7_1213400 ^b	3.5	44.0	(-29 to 76)	0.166	11
PF3D7_1213400	11.8	-88.0	(-342 to 19)	0.132	15
PF3D7_1146100 ^b	5.4	14.4	(-92 to 65)	0.682	15
PF3D7_0930000	1.2	80.8	(55 to 92)	0.001	7

Table S5. Results of quantitative real-time polymerase chain reaction to determine RNA copy number of selected putative transmission-blocking antigens in life cycle stages of *Plasmodium falciparum*. A quantitative real-time polymerase chain reaction (qPCR) was performed to quantify transcript abundance of the putative transmission-blocking antigens in the life stages of *P. falciparum*. SPZ: Sporozoite; BS: asynchronous blood stage; GA: gametocyte; MG: oocysts recovered from midguts of *Anopheles stephensi* 2 (D2) or 6 (D6) days following infectious bloodmeal.

Gene	RNA Copy Number in <i>Plasmodium falciparum</i> life stages				
	SPZ	BS	GA	MG	
				D2	D6
PF3D7_0621400 (315-644) [Pf77]	8680	123485	2632017	2791	107926
PF3D7_1453500 (367-655)	10474	9845	3598732	540	18541
PF3D7_1146100	5849	90794	26395	923	15208
PF3D7_0930000	9228	9436	3306023	4420	12964
PF3D7_1210200 (634-849)	14560	19666	3297270	19429	33506
PF3D7_0530800	2123	258729	8808	558	8278
PF3D7_1213400	33593	7239	3288540	15000	53644

PF3D7_1216500

[PfMDV-1]

11315

104215

2140338

544

70835

Table S6. Calculation of minor allele frequencies for *Plasmodium falciparum* proteins Pf77 and PfMDV-1. Amino acid positions with significant minor allele frequencies are listed for both Pf77 and PfMDV-1. Amino acids with the highest minor allele frequencies are indicated in yellow.

Gene	Amino Acid Position	Allele Frequency
PF3D7_0621400 [Pf77]	15	0.005
	164	0.064
	192	0.005
	210	0.005
	239	0.005
	246	0.057
	253	0.005
	283	0.005
	284	0.005
	286	0.028
	287	0.046
	289	0.020
	290	0.011
	291	0.005
	299	0.021
	300	0.011
	302	0.073
303	0.026	

305	0.005
310	0.024
337	0.009
350	0.014
351	0.040
361	0.005
374	0.005
383	0.005
397	0.005
450	0.005
466	0.330
516	0.005
518	0.005
521	0.005
566	0.005
578	0.005
587	0.005
592	0.005
634	0.005

PF3D7_1216500 [PfMDV-1]

5	0.005
27	0.005
34	0.005
35	0.005

98	0.005
102	0.005
109	0.018
141	0.056
177	0.005
178	0.005
181	0.005
182	0.005
184	0.005
190	0.009
192	0.005
