

# Supplemental Material

Design and Evaluation of Chimeric *Plasmodium falciparum* Circumsporozoite  
Protein-Based Malaria Vaccines

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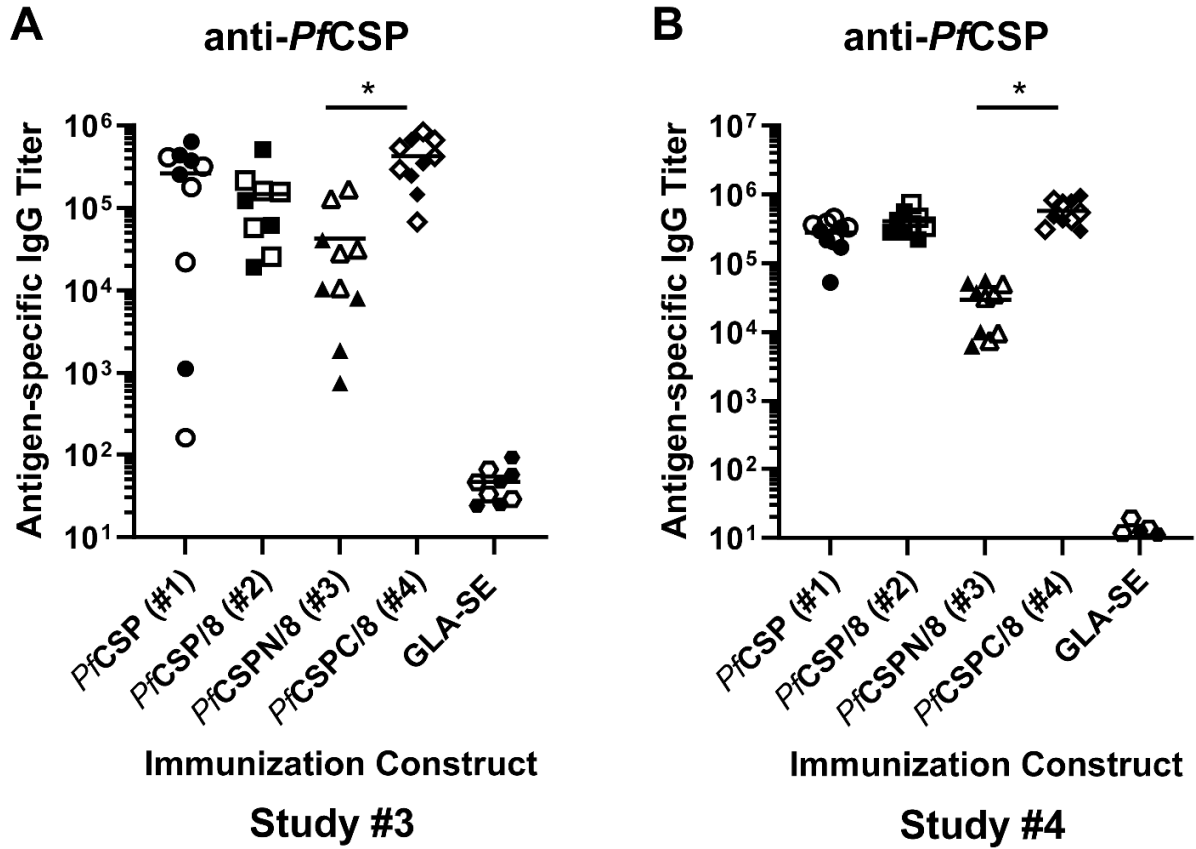
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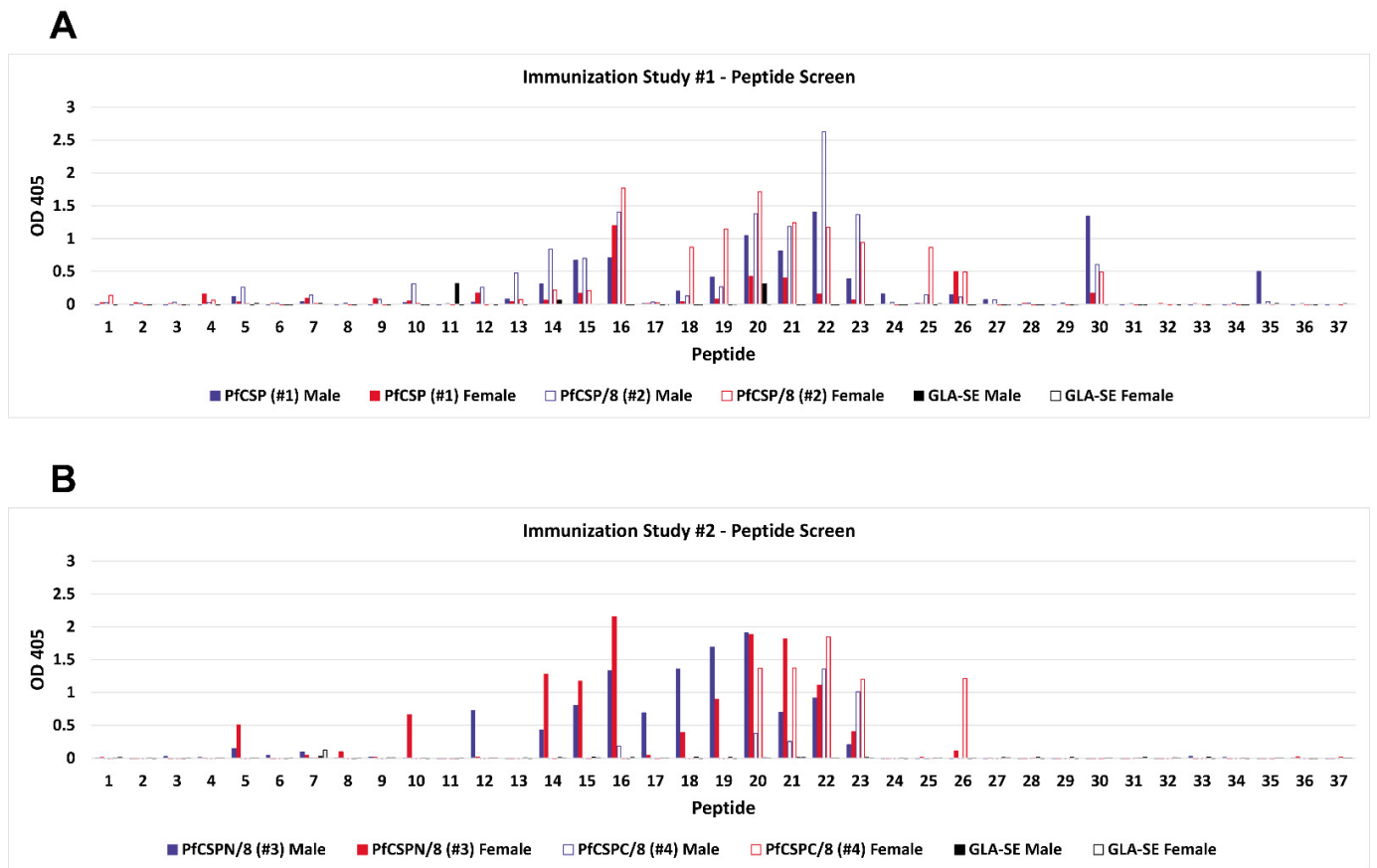
**Table S1**  
**15-mer overlapping peptides of rPfCSP(#1)**

Peptide Number	Peptide Sequence	Peptide Location
1	MAHHHHHHHPGGSGSG	1 - 15
2	HHHPGGSGSGTSGSS	6 - 20
3	SGTSGSSSNTRVLN	13 - 27
4	SSNTRVLNELNYDNA	20 - 34
5	NELNYDNAGTNLYNE	27 - 41
6	AGTNLYNELEMNYYG	34 - 48
7	ELEMNYYGKQENWYS	41 - 55
8	GKQENWYSLKKNSRS	48 - 62
9	SLKKNSRSLGENDDG	55 - 69
10	SLGENDDGNNNNGDN	62 - 76
11	GNNNNGDNGREGKDE	69 - 83
12	NGREGKDEDKRDGNN	76 - 90
13	EDKRDGNNEDNEKLR	83 - 97
14	NEDNEKLRKPKHKKL	90 - 104
15	RKPKHKKLKQPGDGN	97 - 111
16	LKQPGDGNPDNPANP	104 - 118
17	NPDNPANPNVDPNAN	111 - 125
18	PNVDPNANPNVDPNA	118 - 132
19	NPNVDPNANPNVDPN	125 - 139
20	ANPNVDPNANPNANP	132 - 146
21	PNANPLKNANPNANP	146 - 160
22	NANPNANPNANPNAN	Central Repeat
23	NANPNANPNANPNKN	
24	PNANPNKNNQGNGQG	181 - 195
25	NNQGNGQGHNMPNDP	188 - 202
26	GHNMPNDPNRNVDEN	195 - 209
27	PNRNVDENANANNAV	202 - 216
28	NANANNAVKNNNNEE	209 - 223
29	VKNNNNEEPSDKHIE	216 - 230
30	EPSDKHIEQYLKKIK	223 - 237
31*	EQYLKKIKNSISTEW	230 - 244
32	KNSISTEWSPCSVTC	237 - 251
33*	WSPCSVTCGNGIQVR	244 - 258
34*	CGNGIQVRIKPGSAN	251 - 265
35*	RIKPGSANKPKDELD	258 - 272
36*	NKPKDEL DYENDIEK	265 - 279
37*	DYENDIEKKICKMEKCSS	272 - 286
		279 - 296

\* Peptide Pool 6 for T cell assays

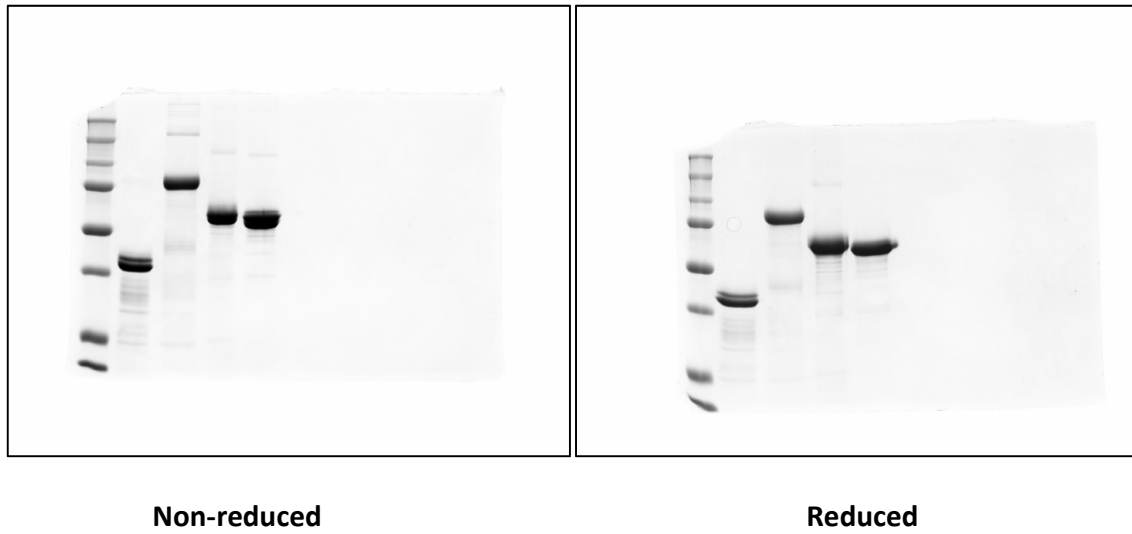


**Figure S1. Magnitude of vaccine-induced antibody responses in immunization studies 3 and 4.** Antigen-specific IgG titers were determined by ELISA on wells coated with *PfCSP* (#1) using secondary sera from CD1 mice (n=10 per group; female, open symbols; male, closed symbols) immunized with *rPfCSP* (#1, circles), *rPfCSP*/8 (#2, squares), *rPfCSPN*/8 (#3, triangles), *rPfCSPC*/8 (#4, diamonds) or GLA-SE (hexagons) in study #3 (panel A) and study #4 (panel B). Statistical significance of differences in titers were assessed by the Kruskal-Wallis test followed by Dunn's correction for multiple comparisons. \*  $p < 0.05$ .

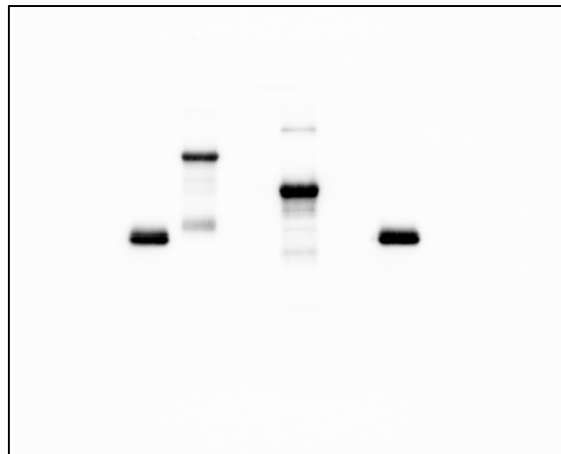


**Figure S2. Initial screen of the specificity of *rPfCSP* vaccine-induced antibodies.** Pools of sera from male (n=5, blue) and female (n=5, red) mice immunized with *rPfCSP* (#1), *rPfCSP/8* (#2), *rPfCSPN/8* (#3), or *rPfCSPC/8* (#4) were assessed by ELISA for reactivity with 37 overlapping 15 amino acid, biotinylated peptides spanning the length of *PfCSP* (#1). Sera from Study #1 is shown in **(A)** and Study #2 is shown in **(B)**. The OD<sub>405</sub> using sera assayed at a dilution of 1:1000 is plotted for each peptide in the array.

**A – Figure 2A, main manuscript**



**B – Figure 2B, main manuscript**



**Figure S3. Assessment of purified *rPfCSP*-based vaccines, original images. (A)** Full images of Figure 2A – Coomassie-blue stained SDS-PAGE gel of purified *rPfCSP*-based vaccines (#1-#4) run under non-reducing (left panel) and reducing (right panel) conditions. **(B)** Full image of Figure 2B immunoblot of purified, non-reduced *rPfCSP*-based vaccines (#1-#4) using mAb 4B3.