

Supplemental Material

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

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

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

* Peptide Pool 6 for T cell assays

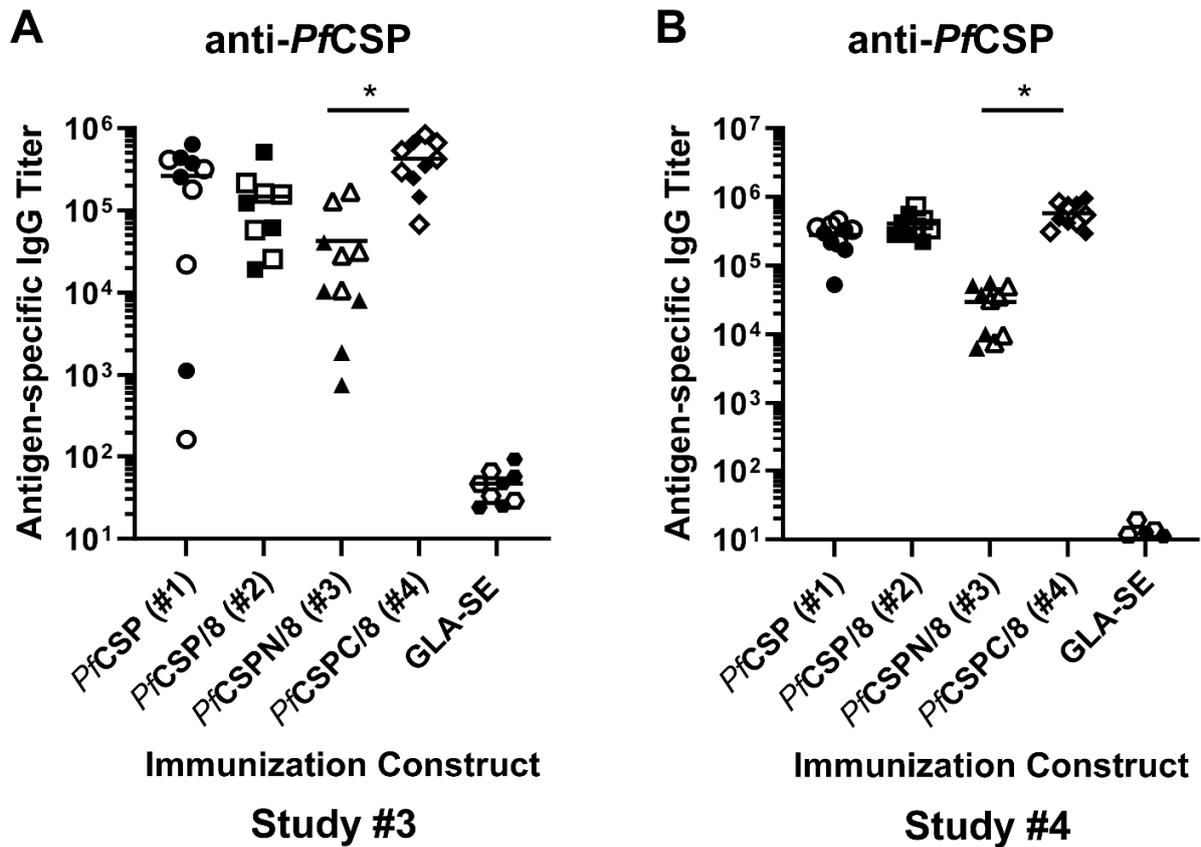


Figure S1. Magnitude of vaccine-induced antibody responses in immunization studies 3 and 4. Antigen-specific IgG titers were determined by ELISA on wells coated with *PfCSP* (#1) using secondary sera from CD1 mice (n=10 per group; female, open symbols; male, closed symbols) immunized with *rPfCSP* (#1, 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. * $p < 0.05$.

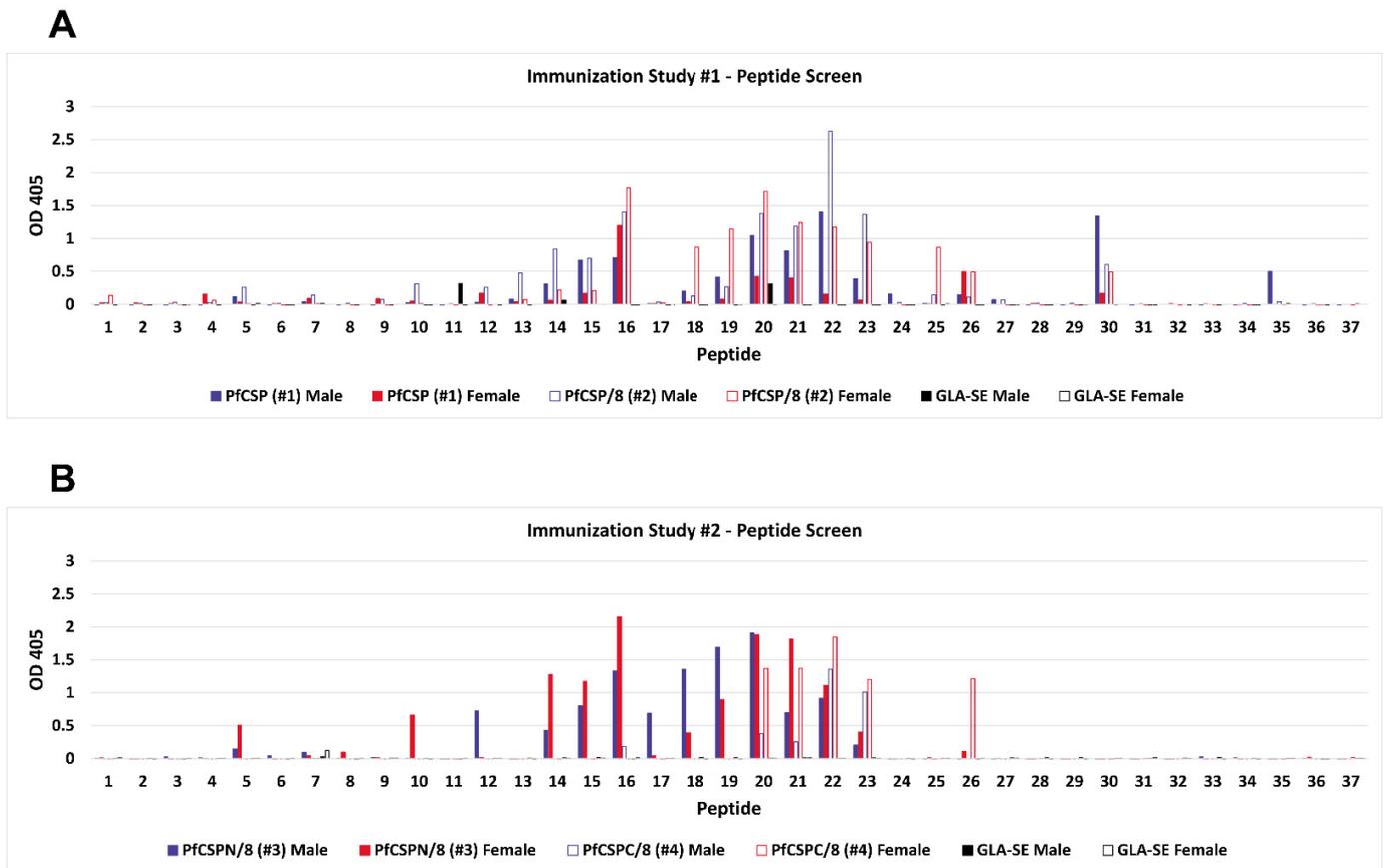
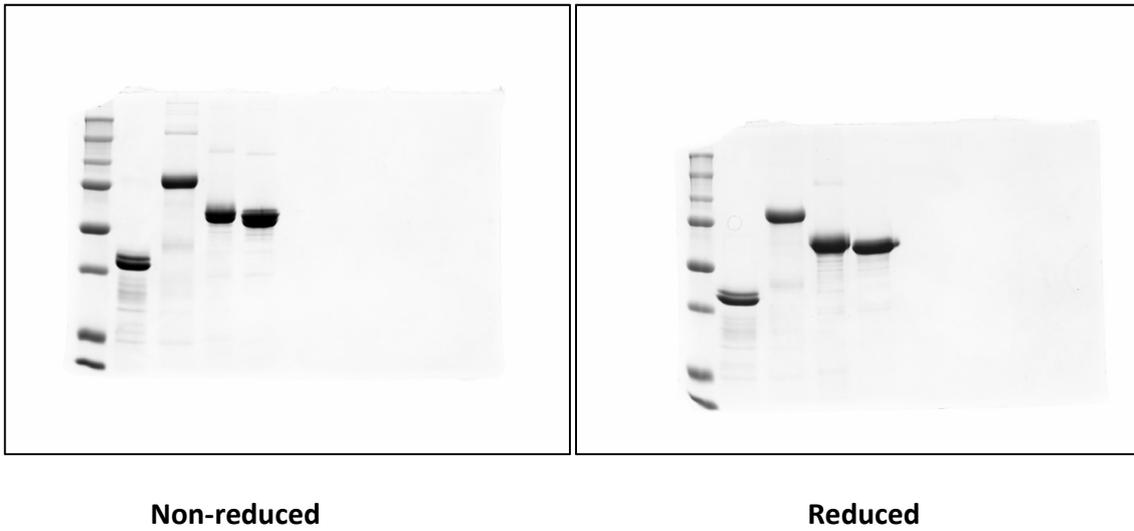


Figure S2. Initial screen of the specificity of *rPfCSP* vaccine-induced antibodies. Pools of sera from male (n=5, blue) and female (n=5, red) mice immunized with *rPfCSP* (#1), *rPfCSP/8* (#2), *rPfCSPN/8* (#3), or *rPfCSPC/8* (#4) were assessed by ELISA for reactivity with 37 overlapping 15 amino acid, biotinylated peptides spanning the length of *PfCSP* (#1). Sera from Study #1 is shown in **(A)** and Study #2 is shown in **(B)**. The OD₄₀₅ 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[t



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.