

Supplementary Information for

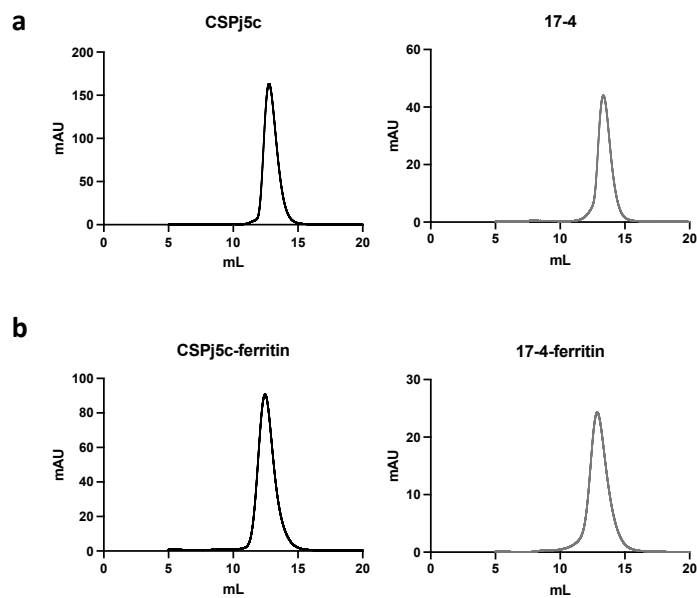
A combined designed CSP and Pfs48/45 infection- and transmission blocking- vaccine for malaria.

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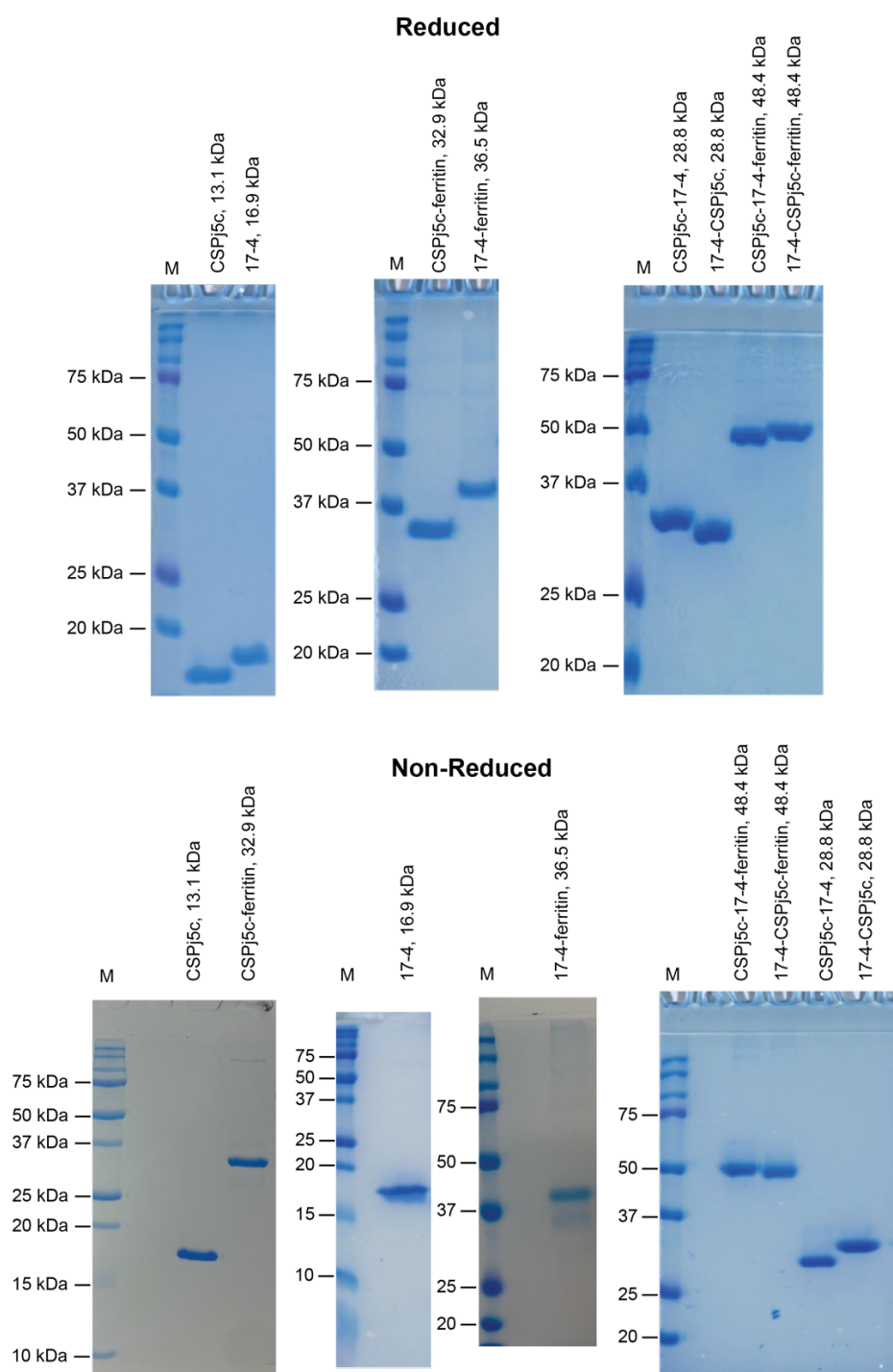
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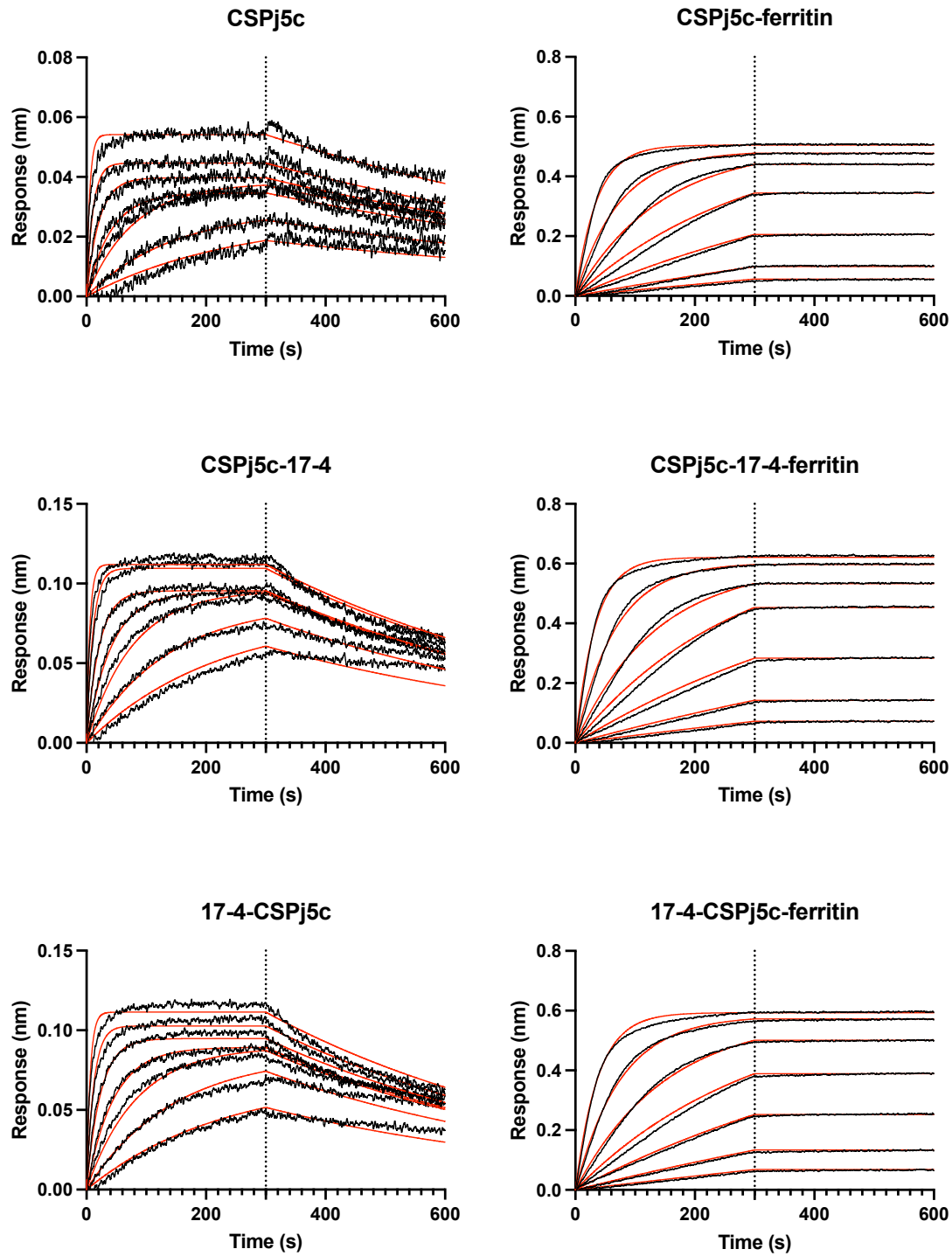
Supplementary Figures 1 to 6
Supplementary Tables 1 to 9



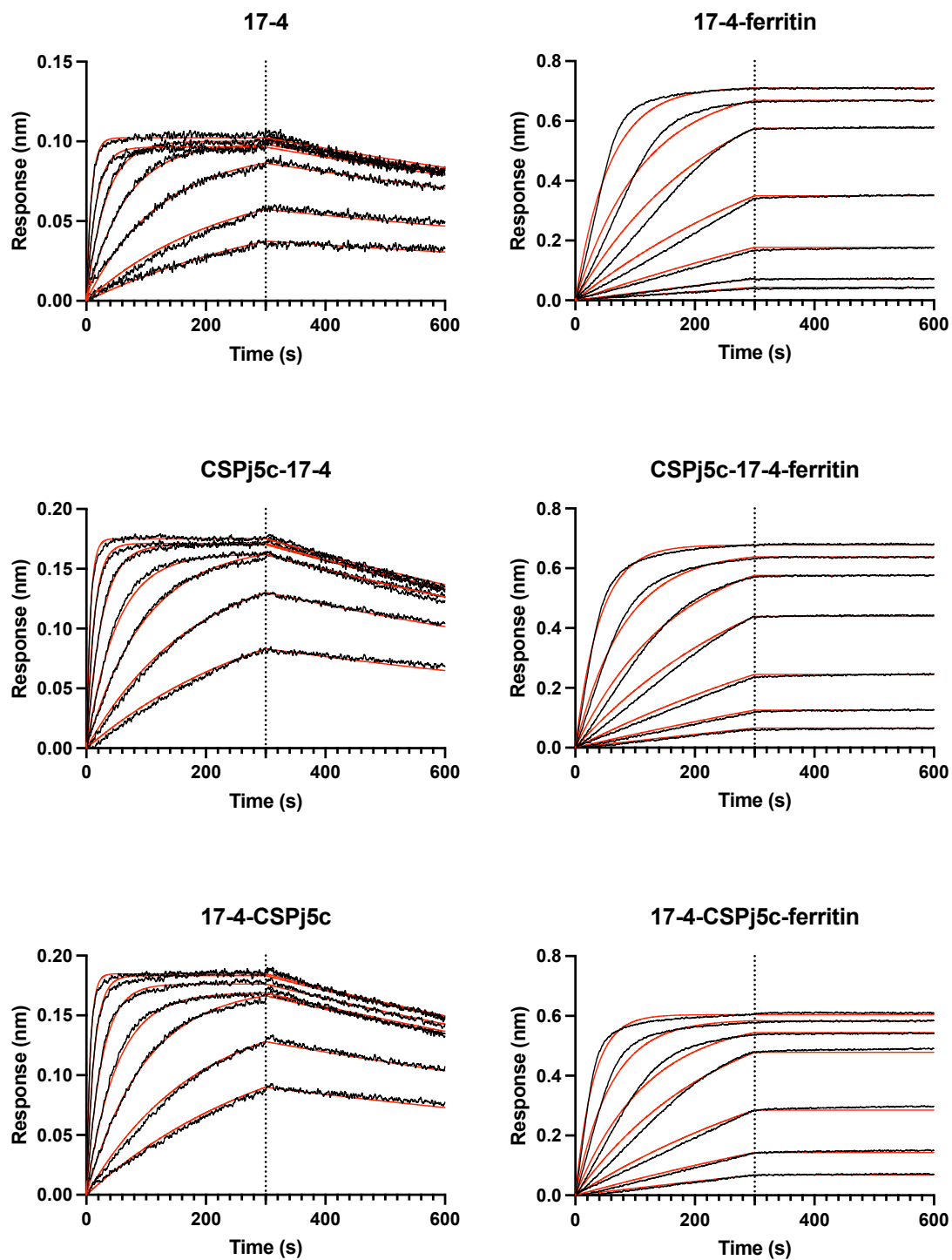
Supplementary Figure 1. a) Size-exclusion chromatography profile of CSPj5c and 17-4 on Superdex 75 Increase 10/300 GL column after nickel resin purification. b) Size-exclusion chromatography profile of CSPj5c-ferritin and 17-4-ferritin on Superose 6 Increase 10/300 GL column from Expi293F supernatant.



Supplementary Figure 2. Purified proteins on SDS-PAGE. Approximately 2 μ g of purified protein was separated on a 12% or 15% Tris-Glycine polyacrylamide gels under reducing or non-reducing conditions and were visualized with coomassie staining. Precision Plus protein marker (M) is used as standard and the sizes (kDa) of the molecular mass markers are indicated.

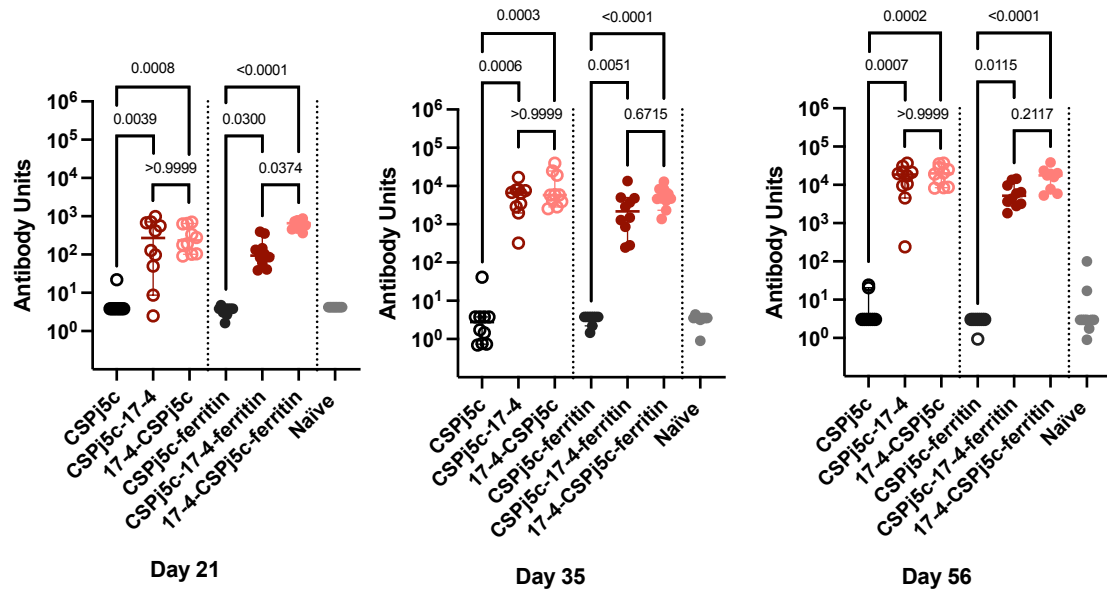


Supplementary Figure 3. Representative BLI traces of CIS43 mAb binding to antigen designs. Two-fold dilution starting from 125 nM of antigen. Raw sensorgrams and fitting curves are shown in black and red, respectively. Association and dissociation phases are divided by the dotted lines.

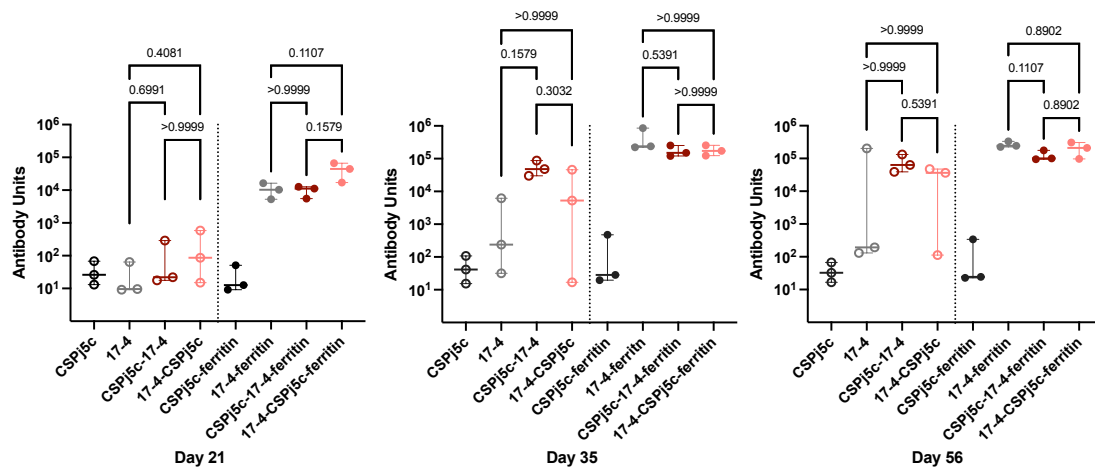


Supplementary Figure 4. Representative BLI traces of TB31F mAb binding to antigen designs. Two-fold dilution starting from 125 nM of antigen. Raw sensorgrams and fitting curves are shown in black and red, respectively. Association and dissociation phases are divided by the dotted lines.

C57BL



Supplementary Figure 5. Antibody IgG titer against Pfs48/45 WT D3 in C57BL/6 immunized mice at days 21, 35 and 56. Median values and 95% confidence levels are displayed with individual values, and the p-values were calculated using the Kruskal-Wallis non-parametric test followed by Dunn's test corrected for the three comparisons in each section of the graph



Supplementary Figure 6. Antibody IgG titer against Pfs48/45 WT D3 in immunized rabbits at days 21, 35 and 56. Median and 95% CI values are displayed. The p-values were obtained using Kruskal-Wallis non-parametric test followed by Dunn's multiple comparisons.

Supplementary Table 1. Sequences for antigen designs.

Pfs48/45 17-4	ETGEKKVIHGCNFASNVASKHTFTKSLDISLVDDSAHISCNVHLSEKKYNHLVG MNCPGDIIPDCFFQVYQQESEEELEPSNIVYLD SQINIGDIEYYEDAEGDDKIKLFKI VGSIPKTTSFTCICKKD KKSAYMTVTIDSAGG TKHHHHHH
CSPj5c	ETGNPDPNANPNVDPNANPNANPNANPNANPNANPNANPNANPNANPNANAEPSDKHIKEYLNKI QNS LSTEWSPCSVTCGNGIQVRIKPGSANKPKDEL DYANDIEKKICKMEKCSSVFNVV NSGT KHHHHHH
17-4-CSPj5c	ETGEKKVIHGCNFASNVASKHTFTKSLDISLVDDSAHISCNVHLSEKKYNHLVG MNCPGDIIPDCFFQVYQQESEEELEPSNIVYLD SQINIGDIEYYEDAEGDDKIKLFKI VGSIPKTTSFTCICKKD KKSAYMTVTIDSAGNPDPNANPNVDPNANPNANPNANPN NANPNANPNANAEPSDKHIKEYLNKI QNSLSTEWSPCSVTCGNGIQVRIKPGSANKP KDEL DYANDIEKKICKMEKCSSVFNVVN SGGSGTKHHHHHH
CSPj5c-17-4	ETGNPDPNANPNVDPNANPNANPNANPNANPNANPNANPNANPNANPNANAEPSDKHIKEYLNKI QNS LSTEWSPCSVTCGNGIQVRIKPGSANKPKDEL DYANDIEKKICKMEKCSSVFNVV NSGGSEKKVIHGCNFASNVASKHTFTKSLDISLVDDSAHISCNVHLSEKKYNHLV GMNCPGDIIPDCFFQVYQQESEEELEPSNIVYLD SQINIGDIEYYEDAEGDDKIKLFK IVGSIPKTTSFTCICKKD KKSAYMTVTIDSAGG TKHHHHHH
17-4-ferritin	ETGEKKVIHGCNFASNVASKHTFTKSLDISLVDDSAHISCNVHLSEKKYNHLVG MNCPGDIIPDCFFQVYQQESEEELEPSNIVYLD SQINIGDIEYYEDAEGDDKIKLFKI VGSIPKTTSFTCICKKD KKSAYMTVTIDSAGGTGSGGGGESQVRQQFSKDIEKLL NEQVNKEMQSSNL YMSMSSWCYTHSLDGAGLFLFDHAAEEYEHA KKLIIFLNE NNVPVQLT SISAPEHKFEGLTQIFQKAYEHEQHISESINNIVDHA IKS KD HATFNFL QWYVAEQHEEEVL FDKDILDKIELIGNENHGLYLADQYVKGI AKSRKSGS
CSPj5c-ferritin	ETGNPDPNANPNVDPNANPNANPNANPNANPNANPNANPNANPNANPNANAEPSDKHIKEYLNKI QNS LSTEWSPCSVTCGNGIQVRIKPGSANKPKDEL DYANDIEKKICKMEKCSSVFNVV NSGGSGTGSGGGGESQVRQQFSKDIEKLLNEQVNKEMQSSNL YMSMSSWCYTH SLDGAGLFLFDHAAEEYEHA KKLIIFLNENNVPVQLT SISAPEHKFEGLTQIFQKA YEHEQHISESINNIVDHA IKS KD HATFNFLQWYVAEQHEEEVL FDKDILDKIELIGN ENHGLYLADQYVKGI AKSRKSGS
17-4-CSPj5c-ferritin	ETGEKKVIHGCNFASNVASKHTFTKSLDISLVDDSAHISCNVHLSEKKYNHLVG MNCPGDIIPDCFFQVYQQESEEELEPSNIVYLD SQINIGDIEYYEDAEGDDKIKLFKI VGSIPKTTSFTCICKKD KKSAYMTVTIDSAGNPDPNANPNVDPNANPNANPNANPN NANPNANPNANAEPSDKHIKEYLNKI QNSLSTEWSPCSVTCGNGIQVRIKPGSANKP KDEL DYANDIEKKICKMEKCSSVFNVVN SGGSGTGSGGGGESQVRQQFSKDIEK LLNEQVNKEMQSSNL YMSMSSWCYTHSLDGAGLFLFDHAAEEYEHA KKLIIFLN ENNVPVQLT SISAPEHKFEGLTQIFQKAYEHEQHISESINNIVDHA IKS KD HATFNF LQWYVAEQHEEEVL FDKDILDKIELIGNENHGLYLADQYVKGI AKSRKSGS
CSPj5c-17-4-ferritin	ETGNPDPNANPNVDPNANPNANPNANPNANPNANPNANPNANPNANPNANAEPSDKHIKEYLNKI QNS LSTEWSPCSVTCGNGIQVRIKPGSANKPKDEL DYANDIEKKICKMEKCSSVFNVV NSGGSEKKVIHGCNFASNVASKHTFTKSLDISLVDDSAHISCNVHLSEKKYNHLV GMNCPGDIIPDCFFQVYQQESEEELEPSNIVYLD SQINIGDIEYYEDAEGDDKIKLFK IVGSIPKTTSFTCICKKD KKSAYMTVTIDSAGGTGSGGGGESQVRQQFSKDIEKLL NEQVNKEMQSSNL YMSMSSWCYTHSLDGAGLFLFDHAAEEYEHA KKLIIFLNE NNVPVQLT SISAPEHKFEGLTQIFQKAYEHEQHISESINNIVDHA IKS KD HATFNFL QWYVAEQHEEEVL FDKDILDKIELIGNENHGLYLADQYVKGI AKSRKSGS

Supplementary Table 2. Median titers in C57BL/6 mice over time. p-values relative to naïve control are displayed in parentheses and were calculated by Kruskal-Wallis followed by Dunn's test corrected for 6 comparisons to the naïve group

Sample Name	CSPj5c Titers			17-4 Titers		
	Day 21	Day 35	Day 56	Day 21	Day 35	Day 56
CSPj5c	2.700 (>0.9999)	3.883 (>0.9999)	2.700 (>0.9999)	4.244 (>0.9999)	1.983 (>0.9999)	3.283 (>0.9999)
CSPj5c-17-4	1937 (0.0004)	97417 (<0.0001)	87667 (<0.0001)	2339 (0.0359)	60533 (0.0004)	113883 (0.0095)
17-4-CSPj5c	3195 (0.0002)	86000 (<0.0001)	130400 (<0.0001)	1766 (0.0237)	61250 (<0.0001)	141350 (0.0007)
CSPj5c-ferritin	49.47 (0.1370)	58.67 (0.2055)	4693 (0.2887)	4.244 (>0.9999)	1.233 (>0.9999)	3.444 (>0.9999)
CSPj5c-17-4-ferritin	7912 (<0.0001)	42467 (<0.0001)	91583 (<0.0001)	2103 (0.0589)	15917 (0.0366)	52067 (0.2703)
17-4-CSPj5c-ferritin	7462 (<0.0001)	46167 (<0.0001)	41333 (0.009)	5140 (0.0002)	38550 (0.0026)	70667 (0.0410)

Sample Name	Pfs48/45 WT Titers			Ferritin Titers		
	Day 21	Day 35	Day 56	Day 21	Day 35	Day 56
CSPj5c	3.833 (>0.9999)	2.767 (>0.9999)	3.067 (>0.9999)	3.417	1.911	3.300
CSPj5c-17-4	271.8 (0.0985)	6627 (0.0001)	18522 (<0.0001)	1.461	2.482	6.583
17-4-CSPj5c	234.5 (0.0269)	5815 (<0.0001)	19833 (<0.0001)	1.550	2.983	6.583
CSPj5c-ferritin	3.833 (0.8129)	3.800 (>0.9999)	3.067 (>0.9999)	3.100 (>0.9999)	2.822 (>0.9999)	48.67 (0.2474)
CSPj5c-17-4-ferritin	93.83 (0.3608)	2183 (0.0084)	5183 (0.0055)	28 (0.0152)	2517 (0.0030)	18312 (<0.0001)
17-4-CSPj5c-ferritin	658.3 (0.0003)	4706 (0.0001)	17770 (<0.0001)	135.2 (0.0001)	8445 (<0.0001)	33100 (<0.0001)

Supplementary Table 3. Geometric mean titers in C57BL/6 mice over time.

Sample Name	CSPj5c Titers			17-4 Titers		
	Day 21	Day 35	Day 56	Day 21	Day 35	Day 56
CSPj5c	2.015	3.889	8.092	5.052	2.526	4.427
CSPj5c-17-4	1086	74673	96130	897.5	40080	86763
17-4-CSPj5c	2511	79824	153934	1973	59702	120005
CSPj5c-ferritin	52.03	62.26	3671	4.863	1.638	3.182
CSPj5c-17-4-ferritin	7803	34108	62678	1919	18668	49319
17-4-CSPj5c-ferritin	7964	42215	41695	5492	33610	72491

Sample Name	Pfs48/45 WT Titers			Ferritin Titers		
	Day 21	Day 35	Day 56	Day 21	Day 35	Day 56
CSPj5c	4.563	2.475	4.559	3.460	2.012	3.792
CSPj5c-17-4	130.9	4317	10677	1.926	2.836	4.050
17-4-CSPj5c	248.5	7330	17676	1.638	3.232	5.952
CSPj5c-ferritin	3.394	3.217	2.687	2.504	2.812	118.6
CSPj5c-17-4-ferritin	106.5	1819	5351	32.07	1887	13127
17-4-CSPj5c-ferritin	609.2	4872	14271	124.9	8252	25309

Supplementary Table 4. Kaplan-Maier analysis and Log-rank (Mantel-Cox) test p-values for comparison with naïve mice post-challenge with PbPfCSP SPZ. The p-values in red indicate significance below Bonferroni corrected threshold ($0.05/6 = 0.0083$).

Sample Name	Log-rank (Mantel-Cox) test p-values
CSPj5c	0.6232
CSPj5c-17-4	0.0052
17-4-CSPj5c	0.0131
CSPj5c-ferritin	0.0849
CSPj5c-17-4-ferritin	0.0025
17-4-CSPj5c-ferritin	0.0014

Supplementary Table 5. Median titers in rabbits over time. p-values relative to a negative control (17-4 for CSPj5c and ferritin titers and CSPj5c for 48/45 titers) are displayed in parentheses and were calculated by Kruskal-Wallis followed by Dunn's test corrected for 6 comparisons to the negative group

Sample Name	CSPj5c Titers			17-4 Titers		
	Day 21	Day 35	Day 56	Day 21	Day 35	Day 56
CSPj5c	403.0 (>0.9999)	5013 (>0.9999)	9960 (>0.9999)	50.67	47.10	37.60
17-4	36.47	80.33	185.3	14.90 (>0.9999)	263.3 (>0.9999)	1057 (>0.9999)
CSPj5c-17-4	1603 (>0.9999)	331867 (0.1517)	299600 (0.6000)	26.63 (>0.9999)	77733 (>0.9999)	110800 (0.6859)
17-4-CSPj5c	1027 (>0.9999)	47160 (>0.9999)	98000 (>0.9999)	30.13 (>0.9999)	18467 (>0.9999)	70667 (>0.9999)
CSPj5c-ferritin	47733 (0.1517)	349333 (0.1517)	499600 (0.0617)	19.47	43.80	49.93
17-4-ferritin	116.1	154.3	168.0	40800 (0.3926)	848533 (0.0095)	824800 (0.0029)
CSPj5c-17-4-ferritin	407167 (0.0037)	1497867 (0.0048)	1361067 (0.0095)	21033 (>0.9999)	403067 (0.0895)	259333 (0.1071)
17-4-CSPj5c-ferritin	136633 (0.0280)	251600 (0.1517)	430400 (0.1517)	118850 (0.1071)	360067 (0.0745)	290800 (0.0419)

Sample Name	Pfs48/45 WT Titers			Ferritin Titers		
	Day 21	Day 35	Day 56	Day 21	Day 35	Day 56
CSPj5c	26.17	41.57	32.37	101.4	132.9	105.8
17-4	9.567 (>0.9999)	238.3 (>0.9999)	193.3 (>0.9999)	57.63	79.33	103.6
CSPj5c-17-4	22.10 (>0.9999)	47867 (0.8866)	63733 (0.5228)	121.0	252.8	218.3
17-4-CSPj5c	86.67 (>0.9999)	5287 (>0.9999)	36683 (>0.9999)	103.1	122.1	99.90
CSPj5c-ferritin	12.73	28.20	24.50	12.73 (>0.9999)	207467 (>0.9999)	740267 (0.0705)
17-4-ferritin	10297 (0.4539)	239033 (0.0148)	244567 (0.0037)	22597 (0.0423)	540533 (0.0548)	332000 (0.2209)
CSPj5c-17-4-ferritin	11160 (0.3926)	149833 (0.0895)	100767 (0.1795)	8280 (0.3313)	483600 (0.1138)	491600 (0.2209)
17-4-CSPj5c-ferritin	44487 (0.0419)	171800 (0.0510)	209867 (0.0280)	18183 (0.0186)	792267 (0.0423)	694533 (0.1784)

Supplementary Table 6. Geometric mean titers in rabbits over time.

Sample Name	CSPj5c Titers			17-4 Titers		
	Day 21	Day 35	Day 56	Day 21	Day 35	Day 56
CSPj5c	500.2	2775	12988	40.92	44.08	40.72
17-4	36.13	108.4	768.4	20.31	407.4	4084
CSPj5c-17-4	1023	304059	249143	50.41	94047	120638
17-4-CSPj5c	794.0	10488	32526	59.04	3803	11995
CSPj5c-ferritin	52937	227227	621551	16.35	45.70	51.05
17-4-ferritin	63.22	281.1	214.0	46427	916907	756784
CSPj5c-17-4-ferritin	312412	1150152	1058939	24241	337394	221921
17-4-CSPj5c-ferritin	126661	344223	416629	113867	456662	344505

Sample Name	Pfs48/45 WT Titers			Ferritin Titers		
	Day 21	Day 35	Day 56	Day 21	Day 35	Day 56
CSPj5c	28.63	41.11	33.02	83.96	108.0	85.58
17-4	17.82	360.3	1726	79.38	93.64	87.22
CSPj5c-17-4	48.48	50111	69193	131.0	199.4	223.6
17-4-CSPj5c	91.58	1602	5817	88.59	186.9	115.0
CSPj5c-ferritin	18.13	63.93	57.50	2849	125557	634225
17-4-ferritin	9598	357318	263610	18942	513861	463445
CSPj5c-17-4-ferritin	9198	166288	118796	8973	476356	508248
17-4-CSPj5c-ferritin	36956	175831	184592	24184	467840	472339

Supplementary Table 7. Transmission reducing activity and the 95% confidence interval values.

	Sample Name	IgG conc [μg/mL]	% Inhibition			
			Estimate	95% CI Low	95% CI High	p-value
SMFA #392-2	CSPj5c	1,000	-74.4	-267.0	21.8	0.173
		333	-51.1	-230.8	32.9	0.322
	17-4	1,000	63.6	18.6	86.1	0.013
		333	14.1	-97.6	63.1	0.722
	CSPj5c-17-4	1,000	53.0	-8.9	80.9	0.070
		333	-22.7	-160.4	47.2	0.636
	17-4-CSPj5c	1,000	12.8	-93.0	61.3	0.722
		333	35.5	-39.3	71.2	0.266
	CSPj5c-ferritin	1,000	40.6	-25.8	72.4	0.172
		333	7.7	-104.1	59.9	0.846
	17-4-ferritin	1,000	100.0	98.3	100.0	0.001
		333	100.0	99.0	100.0	0.001
SMFA #386-2	CSPj5c	1,000	19.7	-83.1	65.8	0.630
		1,000	93.2	81.4	98.8	0.001
	17-4	1,000	98.3	94.8	99.7	0.001
		1,000	41.9	-60.0	83.6	0.253
	CSPj5c-17-4	1,000	45.3	-30.9	80.1	0.158
		1,000	100.0	96.1	100.0	0.001
	17-4-ferritin	1,000	100.0	93.5	100.0	0.001
		1,000	100.0	96.9	100.0	0.001
SMFA #420	CSPj5c-ferritin	333	15.4	-73.5	61.2	0.639
		333	100.0	98.8	100.0	0.0001
	17-4-ferritin	111	74.1	43.3	88.4	0.002
		37	46.3	-16.6	76.3	0.102
		333	87.0	69.4	94.9	0.001
	CSPj5c-17-4-ferritin	111	40.1	-29.9	73.4	0.215
		37	20.4	-76.8	65.4	0.565
		333	92.6	82.3	97.9	0.001
	17-4-CSPj5c-ferritin	111	75.3	39.8	91.3	0.004
		37	50.6	-16.3	80.6	0.094
		333	92.6	82.3	97.9	0.001
		111	75.3	39.8	91.3	0.004
		37	50.6	-16.3	80.6	0.094

Supplementary Table 8. Significance values for comparisons between monomer and the ferritin nanoparticle at each concentration of IgG. The statistical significance values were evaluated by the zero-inflated negative binomial (ZINB) model, and Bonferroni corrected p-values are shown. N/A represents comparisons where statistical analysis was not performed. The statistical analysis was conducted using data from two independent feeds at 1,000 µg/mL and one feed (SMFA#392-2) at 333 µg/mL.

Sample Name	IgG conc. [µg/mL]	CSPj5c-ferritin	17-4-ferritin	CSPj5c-17-4-ferritin	17-4-CSPj5c-ferritin
CSPj5c	333	0.2329	N/A	N/A	N/A
	1,000	0.0260	N/A	N/A	N/A
17-4	333	N/A	0.0010	N/A	N/A
	1,000	N/A	0.0010	N/A	N/A
CSPj5c-17-4	333	N/A	N/A	0.0010	N/A
	1,000	N/A	N/A	0.0010	N/A
17-4-CSPj5c	333	N/A	N/A	N/A	0.0010
	1,000	N/A	N/A	N/A	0.0010

Supplementary Table 9. Significance values for comparisons within monomers and within ferritin nanoparticles at each concentration of IgG. The statistical significance values were evaluated by the zero-inflated negative binomial (ZINB) model, and Bonferroni corrected p-values are shown. N/A represents comparisons where statistical analysis was not performed. The statistical analysis was conducted using data from two independent feeds at 1,000 µg/mL and one feed (SMFA#392-2) at 333 µg/mL.

IgG conc.		333 µg/mL	1000 µg/mL
Sample Name		p-value	p-value
17-4	17-4-CSPj5c	1.000	0.003
17-4	CSPj5c-17-4	1.000	0.546
17-4-CSPj5c	CSPj5c-17-4	0.339	0.003
17-4-ferritin	17-4-CSPj5c-ferritin	0.003	N/A
17-4-ferritin	CSPj5c-17-4-ferritin	0.003	N/A
17-4-CSPj5c-ferritin	CSPj5c-17-4-ferritin	0.006	N/A