

1 **SUPPLEMENTARY INFORMATION**

2 **Supplemental Table 1: IC<sub>50</sub> values of the CspZ-YA IgGs tested in this study for blocking FH binding to CspZ from *B. burgdorferi***  
 3 **B31-5A4.**

IC <sub>50</sub> (nM)								
Rabbit IgG								
Irr. rab. IgG <sup>a</sup>	CspZ-YA IgG (Total)		CspZ-YA IgG (Non-FH-binding sites)			CspZ-YA IgG (FH-binding sites)		
ni. <sup>b</sup>	19.17±2.87		ni.			4.20±0.63		
Mouse IgG								
Irr. ms. IgG <sup>c</sup>	142	224	582	605	651	1009	1139	1193
ni.	82.02±10.12	ni.	ni.	57.90±1.85	97.69±8.94	ni.	4.20±0.72	4.07±0.59

4 <sup>a</sup>Irrelevant rabbit IgG, anti-green fluorescence protein of rabbit IgG.

5 <sup>b</sup>No FH binding inhibition was detected after incubation with 50nM of indicated IgG (the maximal IgG dose used in this study).

6 <sup>c</sup>Irrelevant mouse IgG, anti-green fluorescence protein of mouse IgG.

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12 **Supplemental Table 2: BA<sub>50</sub> values of the CspZ-YA IgGs used in this study**

Mixed with	BA <sub>50</sub> (nM)								
	Rabbit IgG								
	Irr. rab. IgG <sup>a</sup>	CspZ-YA IgG (Total)			CspZ-YA IgG (Non-FH-binding sites)		CspZ-YA IgG (FH-binding sites)		
<i>Bb</i> B31-5A4 <sup>b</sup>	nk. <sup>c</sup>	7.40±0.20			153.30±9.01		2.56±0.08		
<i>Bb</i> 297 <sup>d</sup>	nk.	12.85±0.43			nd. <sup>e</sup>		3.03±0.31		
<i>Ba</i> VS461 <sup>f</sup>	nk.	7.94±1.07			nd.		5.48±0.71		
Mixed with	Mouse IgG								
	Irr. ms. IgG <sup>g</sup>	142	224	582	605	651	1009	1139	1193
<i>Bb</i> B31-5A4	nk.	82.02±10.12	nk.	nk.	57.90±1.85	97.69±8.94	nk.	3.45±0.06	1.23±0.01
<i>Bb</i> 297	nk.	nd.	nd.	nd.	nd.	nd.	nd.	2.48±0.12	1.27±0.02
<i>Ba</i> VS461	nk.	nd.	nd.	nd.	nd.	nd.	nd.	7.13±0.53	51.60±1.92

13 <sup>a</sup>Irrelevant rabbit IgG, anti-green fluorescence protein of rabbit IgG.

14 <sup>b</sup>*B. burgdorferi* strain B31-5A4

15 <sup>c</sup>No killing was detected after incubation with 50nM of indicated IgG (the maximal IgG dose used in this study).

16 <sup>d</sup>*B. burgdorferi* strain 297

17 <sup>e</sup>Not determined

18 <sup>f</sup>*B. afzelii* strain VS461

19 <sup>g</sup>Irrelevant mouse IgG, anti-green fluorescence protein of mouse IgG.

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36 **Supplemental Table 3: Strains and plasmids used in this study.**

Strain or plasmid	Genotype or characteristic	Source
<u><i>B. burgdorferi</i></u>		
B31-5A4	Clone 5A4 of <i>B. burgdorferi</i> B31 isolated from <i>I. scapularis</i> ticks in US.	(1)
297	Clone A11/B11 of <i>B. burgdorferi</i> 297 isolated from human Cerebrospinal fluid from US.	(2, 3)
<u><i>B. afzelii</i></u>		
VS461	Clone JL of <i>B. afzelii</i> VS461 isolated from <i>I. ricinus</i> ticks in Switzerland.	(4)
<u><i>E. coli</i></u>		
BL21(DE3)	F <sup>-</sup> , <i>ompT hsdSB</i> (rB <sup>-</sup> mB <sup>-</sup> ) <i>gal dcm</i> (DE3)	Novagene
BL21(DE3)/pET41a-CspZ	BL21(DE3) producing residues 19 to 237 of CspZ followed by a TEV protease cleavage site and hexa-histidine	This study
BL21(DE3)/pET41a-CspZ-YA	BL21(DE3) producing residues 19 to 237 of CspZ-YA followed by a TEV protease cleavage site and hexa-histidine	This study
<u>Plasmids</u>		
pET41a-CspZ	KanR <sup>a</sup> ; pET41a encoding protein residue 19 to 237 of CspZ followed by a TEV protease cleavage site and hexa-histidine	This study
pET41a-CspZ-YA	KanR; pET41a encoding protein residue 19 to 237 of CspZ-YA followed by a TEV protease cleavage site and hexa-histidine	This study

37 <sup>a</sup> Kanamycin resistant

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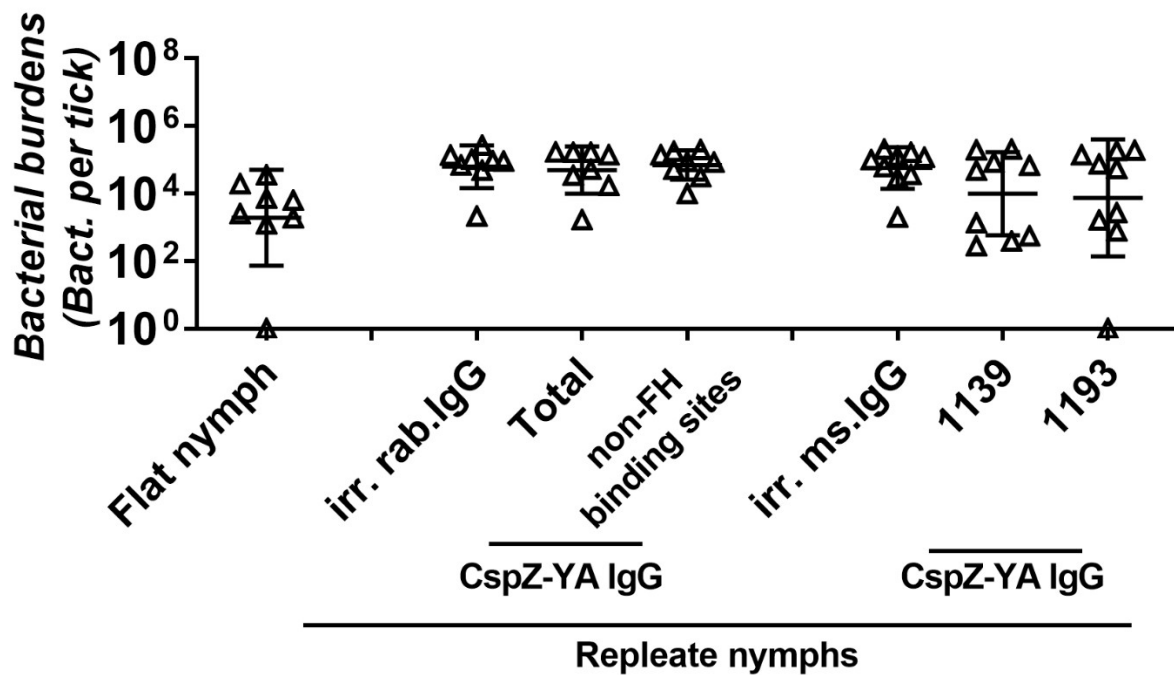
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 46 **Supplemental Figure 1. Passive inoculation of CspZ-YA IgGs does not eliminate *B.***  
 47 ***burgdorferi* B31-5A4 in ticks feeding on mice.** C3H/HeN mice were inoculated with irrelevant  
 48 IgG from rabbits (irr. rab. IgG) or mice (irr. ms. IgG), or CspZ-YA IgG samples (1mg/kg, five  
 49 mice per group). These CspZ-YA IgGs include total CspZ-YA IgG (Total), those IgGs that  
 50 recognize non-FH-binding site (non-FH-binding sites), or mouse monoclonal IgGs #1139 or 1193.  
 51 At 24 hours after IgG inoculation, these mice were fed on by *I. scapularis* nymphs carrying *B.*  
 52 *burgdorferi* B31-5A4 (*Bb* B31-5A4) and those nymphs feeding to repletion were collected. The  
 53 nymphs prior to feeding were also included as control (Flat nymphs). Spirochete burdens at those  
 54 nymphs were quantitatively measured and shown as the number of spirochetes per nymph (Bact.  
 55 per tick). Data shown are the geometric mean  $\pm$  geometric standard deviation of the bacterial  
 56 burdens from eight flat nymphs or the nymphs feeding on mice inoculated with irrelevant rabbit  
 57 IgG, total CspZ-YA IgG, or those IgG that recognize non-FH-binding site, or nine nymphs feeding  
 58 on mice inoculated with irrelevant mouse IgG or the mouse monoclonal antibody #1139 or 1193.

59 No statistical significances ( $p > 0.05$ , Kruskal-Wallis test with the two-stage step-up method of  
60 Benjamini, Krieger, and Yekutieli) of differences in bacterial burdens between the groups of  
61 nymphs were detected.

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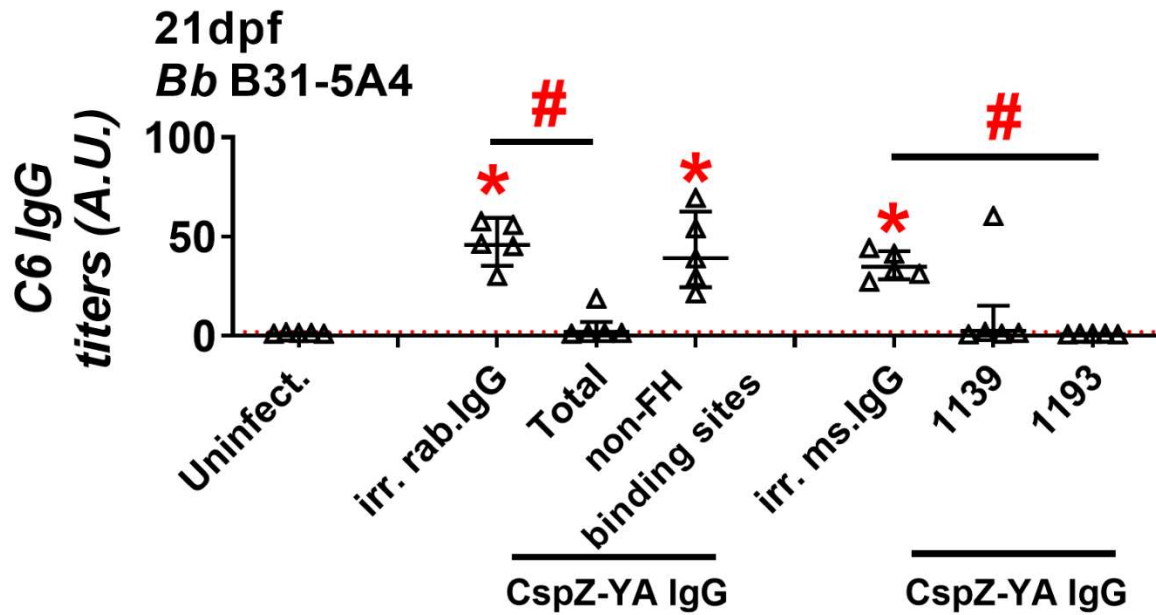
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83 **Supplemental Figure 2. CspZ-YA IgGs that recognize CspZ FH-binding sites selectively**

84 **prevent seropositivity caused by *B. burgdorferi* B31-5A4 infection.** C3H/HeN mice were

85 inoculated with irr. IgG from rabbits (irr. rab. IgG) or mice (irr. ms. IgG), or CspZ-YA IgG samples

86 (1 mg/kg, five mice per group). These CspZ-YA IgGs include total CspZ-YA IgG (Total), those

87 IgGs that recognize non-FH-binding site (non-FH-binding sites), or mouse monoclonal IgG #1139

88 or 1193. At 24 hours after IgG inoculation, these mice were fed on by *I. scapularis* nymphs

89 carrying *B. burgdorferi* B31-5A4 (*Bb* B31-5A4). An additional five mice inoculated with PBS but

90 not fed on by ticks were included as the control (Uninfect.). The sera were collected from those

91 mice at 21dpf. Seropositivity was determined by measuring the levels of IgG against C6 peptides

92 in the sera of those mice were using ELISA. The mouse was considered as seropositive if that

93 mouse had IgG levels against C6 peptides greater than the threshold, the mean plus three-fold

94 standard deviation of the IgG levels against C6 peptides from the PBS-inoculated, uninfected mice

95 (red dotted line). The number of mice in each group with the anti-C6 IgG levels greater than the

96 threshold (seropositive) is shown in Table 1. Data shown are the geometric mean  $\pm$  geometric

97 standard deviation of the titers of anti-C6 IgG. Statistical significances ( $p < 0.05$ , Kruskal-Wallis  
98 test with the two-stage step-up method of Benjamini, Krieger, and Yekutieli) of differences in IgG  
99 titers relative to (\*) uninfected mice or (#) between indicated groups of mice are presented.

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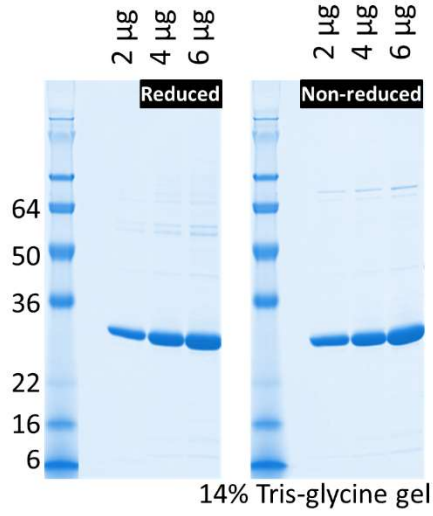
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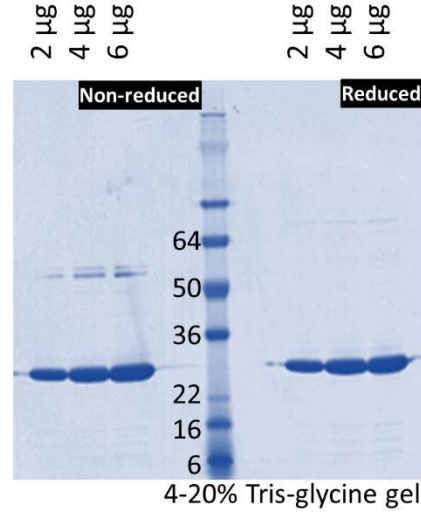


### A CspZ-YA+His



Loading ( $\mu\text{g}$ )	Purity (%)	
	Non-reduced	reduced
2	93.0	93.8
4	93.6	94.0
6	93.1	93.9
<b>Mean</b>	<b>93.2<math>\pm</math>0.3</b>	<b>93.9<math>\pm</math>0.1</b>

### B CspZ+His



Loading ( $\mu\text{g}$ )	Purity (%)	
	Non-reduced	reduced
2	92.4	94.6
4	90.2	95.3
6	93.1	95.5
<b>Mean</b>	<b>91.9<math>\pm</math>1.2</b>	<b>95.1<math>\pm</math>0.4</b>

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121 **Supplemental Figure 3. Purity assessment for the purified his-tagged CspZ-YA and CspZ.**

122 Two to six micrograms of CspZ-YA or CspZ were loaded onto 14% or 4-20% tris-glycine SDS-  
 123 PAGE gels. The purity of each of these proteins was analyzed by densitometry and shown at the  
 124 bottom panels.

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132 **REFERENCES**

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