## *Supplementary Material*

# Eliminating factor H-binding activity of *B. burgdorferi* CspZ combined with virus-like particle conjugation enhances its efficacy as a Lyme disease vaccine

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**Supplementary Figures and Tables**

**Supplementary Figures**

**Supplementary Figure 1. Experimental timeline of mouse immunization and infection.** The timeline to **(A)** generate anti-CspZ serum, **(B)** passive immunization and bacteria challenge, and **(B)** active immunization and bacteria challenge was shown. **(A)** C3H/HeN mice received immunization and two boosters (14 and 28 days post immunization) of VLP, CspZ, VLP-CspZ, or VLP-CspZ-Y207A/Y211A, or PBS. Forty-two days after the initial immunization, sera from these mice were collected to detect their IgG or IgM titers and bactericidal activity. **(B)** Naive SW mice were inoculated with the serum from each group of the mice or pre-immune serum one day before bacteria challenge. These mice were then infected with $10^4$ *B. burgdorferi* strain B31-A3. Tissues collected at 14 days post infection were placed in culture medium to determine the presence of *B. burgdorferi*. **(B)** C3H/HeN mice were received immunization and two boosters (14 and 28 days post immunization) of VLP, CspZ, VLP-CspZ, or VLP-CspZ-Y207A/Y211A, or PBS as shown in panel (A). Forty-two days after initial immunization, those mice were infected with $10^4$ *B. burgdorferi* strain B31-A3. The diameter of the tibiotarsus joints were measured prior to infection as well as 7 and 14 days post infection. Mice were sacrificed 14 days post infection for histopathology, or 28 days post infection for bacterial burden quantification.

**Supplementary Figure 2. Recombinant version of CspZ-Y207A/Y211A is incapable of binding to mouse FH.** The indicated concentrations of GST tagged CspZ ("CspZ") or CspZ-Y207A/Y211A ("CspZ-Y207A/Y211A") or GST were added to triplicate wells coated with 1 μg of BSA (negative control, data not shown) or mouse FH, and protein binding was quantitated by ELISA (see Materials and Methods). The $K_D$ value of CspZ to bind to mouse FH (0.72±0.42μM) was obtained by fitting the binding values to the equation described in Materials and Methods ($R^2 = 0.9463$). Numbers represent the mean ± standard deviation. Data represent the average of four replicates.

**Supplementary Tables**

**Supplementary Table 1. Quantitative determination of borreliacidal activity in the serum obtained from CspZ-, VLP- or PBS-inoculated mice.**

| Vaccination | 50% borreliacidal titer |
|---|---|
| **PBS**[a] | NI[c] |
| **VLP**[b] | NI |
| **CspZ**[b] | 43.02±16.23 |
| **VLP-CspZ**[b] | 143.24±57.85 |
| **VLP-CspZ-Y207A/Y211A**[b] | 395.81±163.72 |

Data shown are mean ± standard error of the mean

[a] Three mice per group

[b] Five mice per group

[c] NI: No inhibition (no killing)

**Supplementary Table 2. *B. burgdorferi* burden in tissues from CspZ-, VLP- or PBS-inoculated mice at 28 days post infection.**

| Immunogen | Colonization (Bacteria/10ng DNA) | | |
|---|---|---|---|
| | **Inoculation Site** | **Joint** | **Heart** |
| **VLP**[a] | 27.54 ± 15.27 | 12.01 ± 1.90 | 15.73 ± 4.15 |
| **CspZ**[a] | 26.92 ± 5.06 | 12.32 ± 3.63 | 15.62 ± 4.34 |
| **VLP-CspZ**[a] | 19.63 ± 2.93 | 2.72 ± 0.35* | 5.77 ± 2.13* |
| **VLP-CspZ-Y207A/Y211A**[b] | 9.30 ± 1.12* | 4.29 ± 0.15* | 2.94 ± 0.95* |

Data shown are mean ± standard deviation of the number of *B. burgdorferi* present as determined from qPCR, based on the data in Figure 6.

[a] Five mice per group

[b] Six mice

* Bacterial burden are below the limit of detection (10 bacteria/10ng DNA), and significantly lower ($p < 0.05$) than VLP-vaccinated mice as determined with one-way ANOVA test and post-hoc analysis.